BIOAVAILABILITY OF VARIOUS VITAMIN E COMPOUNDS IN RUMINANTS

BY

NICHOLAS HIDIROGLOU

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1989

This work is dedicated to my parents,
Dr. and Mrs. Hidiroglou, for their
continual support and encouragement
throughout the doctoral program

ACKNOWLEDGMENTS

The author wishes to express his sincere gratitude to Dr. L. R. McDowell, chairman of the supervisory committee, for his guidance, patience and friendship throughout the doctoral program. The author gratefully acknowledges the assistance and time provided by other members of the supervisory committee, including Drs. Douglas Bates Joseph Conrad, Kermit Bachman and Rachel Shireman.

Deep appreciation is extended to Dr. Keith U. Ingold of the National Research Council of Canada (NRCC) for providing laboratory facilities. The author is especially indebted to Dr. Graham W. Burton of the NRCC for his guidance, patience, and friendship. The technical assistance of Ann Webb, Dave Lindsay, Ewa Lusztyk of the NRCC is deeply appreciated. Many special thanks are extended to Dr. L. Arvanitis for his continual support and friendship throughout my graduate program.

Assistance of fellow graduate students, Osvaldo Balbuena, Pablo Cuesta, Edmundo Espinoza, Akhmad Prabowo, Rodrigo Pastrana, Liberdo Ochoa and Roger Merkel, during blood and tissue collections is appreciated. Special gratitude is expressed to Rodrigo Pastrana and Roger Merkel for continual support during tissue collections from animals.

Sincere appreciation is expressed to Mrs. Pat French for typing this dissertation.

TABLE OF CONTENTS

DEDICATION.......... ii

ACKNOWLEDGMENTS.......... iii

LIST OF TABLES.......... vii

LIST OF FIGURES.......... viii

ABSTRACT.......... [illegible]

CHAPTER 1 INTRODUCTION.......... 1

CHAPTER 2 REVIEW OF LITERATURE.......... [illegible]

Chemistry of Vitamin E.......... [illegible]
Absorption and Storage of Vitamin E.......... [illegible]
The Metabolism of Vitamin E.......... [illegible]
Vitamin E Deficiency.......... [illegible]
Biological Activity of Vitamin E.......... [illegible]
Role of Vitamin E in the Respiratory Chain.......... 41
Intervention of Vitamin E in Hematopoiesis.......... 42
Biological Role of Vitamin E.......... 43
Role of α-Tocopherol as a Chain Breaking Antioxidant.......... 49

CHAPTER 3 PLASMA AND TISSUE LEVELS OF VITAMIN E IN SHEEP FOLLOWING INTRAMUSCULAR ADMINISTRATION IN AN OIL CARRIER.......... 51

Introduction.......... 51
Experimental.......... 51
Analytical.......... 52
Statistical Analysis.......... 53
Results.......... 54
Discussion.......... 59
Summary.......... 61

CHAPTER 4 BIOAVAILABILITY OF VARIOUS VITAMIN E COMPOUNDS IN SHEEP.......... 63

Introduction.......... 63
Materials and Methods.......... 63
Results.......... 67
Discussion.......... 76
Summary.......... 77

Page

CHAPTER 5 BLOOD PLASMA AND TISSUE CONCENTRATIONS OF VITAMIN E IN BEEF CATTLE AS INFLUENCED BY SUPPLEMENTATION OF VARIOUS TOCOPHEROL COMPOUNDS 79

- Introduction 79
- Materials and Method 80
- Results 84
- Discussion 91
- Summary 93

CHAPTER 6 PLASMA TOCOPHEROL IN RUMINANTS AFTER INGESTING FREE OR ACETYLATED TOCOPHEROL 94

- Introduction 94
- Materials and Methods 94
- Results 99
- Discussion 102
- Summary 105

CHAPTER 7 PLASMA AND TISSUES VITAMIN E CONCENTRATIONS IN SHEEP AFTER ADMINISTRATION OF A SINGLE INTRAPERITONEAL DOSE OF dl-α-TOCOPHEROL 106

- Introduction 106
- Experimental Procedure 106
- Results 109
- Discussion 117
- Summary 119

CHAPTER 8 GENERAL CONCLUSIONS 120

LITERATURE CITED 124

BIOGRAPHICAL SKETCH 139

LIST OF TABLES

TABLE PAGE

2.1. NOMENCLATURE FOR TOCOPHEROLS.......... 7

2.2 SPECIFIC OPTICAL ROTATIONS OF NATURAL TOCOPHEROLS.......... 10

2.3. RELATIVE ACTIVITY OF VARIOUS ALPHA-TOCOPHEROL STEREOISOMERS AS DETERMINED BY RESORPTION STERILITY TEST IN FEMALE RATS.......... 33

2.4. RELATIVE BIOLOGICAL ACTIVITY OF VITAMIN E COMPOUNDS IN THE IN VITRO HEMOLYSIS TEST, EXCERPT FROM BRUBACHER AND WEISER (1968).......... 36

2.5 RELATIVE BIOLOGICAL ACTIVITY OF TOCOPHEROLS AND THEIR ESTERS WITH ACETIC ACID IN THE IN VITRO/IN VIVO HEMOLYSIS TEST IN RATS; EXCERPT FROM BLISS AND GYORGY (1969).......... 36

2.6 USP WEIGHT/UNIT RELATIONSHIPS OF TOCOPHEROL.......... 41

3.1. D-α-TOCOPHEROL PHARMACOKINETIC PLASMA VALUES IN SHEEP AFTER A SINGLE INTRAMUSCULAR ADMINISTRATION OF VITAMIN E (40 mg/kg).......... 56

3.2. α-TOCOPHEROL LEVELS IN THE TISSUES (μg/g wet) OF SHEEP FOLLOWING A SINGLE INTRAMUSCULAR ADMINISTRATION (40 mg/kg BODY WEIGHT) OF VARIOUS VITAMIN E PREPARATIONS.......... 58

4.1. ANALYSIS OF VARIANCE FOR α-TOCOPHEROL CONCENTRATIONS IN TISSUES OF SHEEP FED DIFFERENT FORMS OF VITAMIN E. 68

4.2 TISSUE α-TOCOPHEROL CONCENTRATIONS (μg/g FRESH TISSUE) IN THE FOUR DIETS.......... 68

4.3 RETENTION TIME OF α-TOCOPHEROL FROM GAS-CHROMATOGRAPHY/MASS SPECTROMETRY.......... 71

4.4. ANALYSIS OF VARIANCE OF α-TOCOPHEROL CONCENTRATIONS IN BLOOD PLASMA OF SHEEP FED DIFFERENT FORMS OF VITAMIN E.......... 74

4.5 AREA (AUC) UNDER THE PLASMA α-TOCOPHEROL CONCENTRATION CURVE (μg/ml h^{-1}).......... 74

TABLE PAGE

5.1. COMPOSITION OF THE GRAIN PORTION OF THE DIET FED TO COWS FOR 28 d 81

5.2. PLASMA CONCENTRATION OF α TOCOPHEROL (μg/ml) IN CATTLE FED VARIOUS PREPARATIONS OF VITAMIN E. 85

5.3 TISSUE α-TOCOPHEROL CONCENTRATIONS (μg/g FRESH TISSUE) IN CATTLE FED VARIOUS VITAMIN E PREPARATIONS. 88

6.1 DL-α TOCOPHEROL PHARMACOKINETIC PLASMA VALUES (LEAST SQUARE MEANS ± SE) IN SHEEP AFTER A SINGLE ORAL ADMINISTRATION OF VITAMIN E (100 mg/kg BODY WEIGHT) . . . 100

6.2 TRIAL 2: D-α-TOCOPHEROL PHARMACOKINETIC PLASMA VALUES (LEAST SQUARE MEANS ± SE) IN CATTLE AFTER A SINGLE ORAL ADMINISTRATION OF VITAMIN E (50 mg/kg BODY WEIGHT) 101

7.1 DIET OF SHEEP 107

7.2. MEAN LEVELS OF α-TOCOPHEROL IN TISSUES (μg/g FRESH) AND PLASMA (μg/ml) AT TIME OF SLAUGHTER. 110

7.3. ANALYSIS OF VARIANCE MEAN SQUARES OF THE LOG TISSUE VALUES 112

7.4. MEANS OF THE RATIO OF TISSUE TO PLASMA LEVELS OF α-TOCOPHEROL AT TIME OF SLAUGHTER 114

7.5. ANALYSIS OF VARIANCE MEAN SQUARES OF THE LOG RATIO OF TISSUE TO PLASMA 115

7.6 PLASMA TOCOPHEROL (μg/ml) VALUES AND SUMMARIES FOR THOSE ANIMALS SLAUGHTERED AT 28 DAYS 116

LIST OF FIGURES

FIGURE		PAGE
2.I	STRUCTURAL FORMULA OF α-TOCOPHEROL.	3
2.2	STRUCTURAL FORMULAS OF TOCOPHEROLS..	4
2.3	STRUCTURAL FORMULAS OF TOCOTRIENOLS	4
2.4	ASYMMETRIC CARBONS OF TOCOPHEROLS.	5
2.5	BIOSYNTHESIS OF THE RING STRUCTURE OF TOCOPHEROLS AND TOCOTRIENOLS..	9
2.6	STRUCTURE OF TOCOPHERYL QUINONE.	I1
2.7	STRUCTURE OF α TOCORED	I2
2.8	STRUCTURE OF α-TOCOPURPLE	I2
2.9	SCHEMATIC REPRESENTATION OF THE PEROXIDATION OF UNSATURATED FATTY ACIDS.	45
3.I	PLASMA α-TOCOPHEROL LEVELS IN SHEEP AFTER SINGLE I.M. INJECTION OF D-α-TOCOPHEROL SUSPENDED IN STERILE SESAME OIL	55
4.I	α-TOCOPHEROL CONCENTRATIONS IN THE VARIOUS TISSUES OF SHEEP SUPPLEMENTED WITH DIFFERENT VITAMIN E COMPOUNDS.	69
4.2	IDENTIFICATION OF α TOCOPHEROL BY GAS-CHROMATOGRAPHY/MASS SPECTROPHOTOMETRY (A) α-TOCOPHEROL STANDARD. (B) α-TOCOPHEROL IN PANCREAS	72
4.3	MASS SPECTRAL SCAN OF THE α TOCOPHEROL STANDARD (A) AND α-TOCOPHEROL IN THE PANCREATIC TISSUE (B) FOLLOWING HPLC COLLECTION	73
4.4	α-TOCOPHEROL CONCENTRATIONS IN THE BLOOD PLASMA OF SHEEP SUPPLEMENTED WITH DIFFERENT VITAMIN E COMPOUNDS.	75
5.I	α-TOCOPHEROL CONCENTRATIONS IN THE TISSUES OF CATTLE SUPPLEMENTED WITH DIFFERENT VITAMIN E COMPOUNDS	87

FIGURE PAGE

5.1 MASS SPECTRAL SCANNING OF THE α-TOCOPHEROL STANDARD (A) AND IN BLOOD PLASMA (B) FOLLOWING HPLC COLLECTION AND TLC 90

6.1 PLASMA TOCOPHEROL CONCENTRATION (μG/ML) IN SHEEP FOLLOWING ADMINISTRATION OF A SINGLE MEGADOSE (100 MG/KG BODY WEIGHT) OF (A) DL-α-TOCOPHERYL ACETATE OR (B) DL-α-TOCOPHEROL 96

6.2 PLASMA TOCOPHEROL CONCENTRATION (μG/ML) IN CATTLE FOLLOWING ADMINISTRATION OF A SINGLE DOSE (50 MG/KG BODY WEIGHT) OF (A) DL-α-TOCOPHERYL ACETATE OR (B) DL-α TOCOPHEROL 97

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

BIOAVAILABILITY OF VARIOUS VITAMIN E COMPOUNDS IN RUMINANTS

By

NICHOLAS HIDIROGLOU

August 1989

Chairman: Dr. L. R. McDowell
Major Department: Animal Science

This research was designed to investigate the bioavailability of various tocopherol sources for ruminants. In experiment I, d-α-tocopherol and dl-α-tocopherol suspended in sesame oil were administered intramuscularly in sheep at a level of 40 mg/kg body weight (b.w.). Sheep administered d-α-tocopherol and killed after 360 hr resulted in a higher ($P<.01$) bioavailability than for sheep injected with d-α-tocopherol or dl-α-tocopherol and sacrificed 240 hr after dosing. A more sustained increment in plasma α-tocopherol was observed with d-α-tocopherol than dl-α-tocopherol.

In experiment II, sheep were provided with 400 IU/d of either d-α-tocopherol, dl-α-tocopherol or their corresponding acetate forms for 28 d. Higher concentrations of α-tocopherol were observed in tissues of sheep fed d-α-tocopherol than the other tocopherol forms. In sheep fed d-α-tocopherol, the area under the plasma α-tocopherol time curve (AUC), was larger as compared to other forms of vitamin E supplementation.

Experiment III investigated the concentration of α-tocopherol in plasma and tissues of beef cows following a daily oral administration of 1000 IU of four tocopherol sources for 28 d. The d-α-tocopherol and its acetate ester increased plasma tocopherol concentrations faster (P<.05) than the racemic products, with the greatest response occurring with d-α-tocopherol. Tissue analyses confirmed that in adrenal gland, kidney, liver and lung, α-tocopherol concentrations were higher (P<.05) following d-α-than dl-α-tocopherol supplementation.

In experiment IV, dl-α-tocopherol and its ester were administered to sheep or cattle (100 or 50 mg/kg b.w., respectively) in a single oral dose. Blood plasma α-tocopherol tolerance curve area was higher (P<.05) in the dl-α tocopherol group than in its ester form, as well as quicker (P<.05) time (h) for maximum plasma α-tocopherol concentration. A greater (P<.05) plasma tolerance curve area was observed in the cattle following administration of dl-α-tocopherol than its acetylated form.

In experiment V plasma and tissue vitamin E concentrations were determined following a 5 g dosage of dl-α-tocopherol intraperitoneally in sheep, which were slaughtered on d 3, 6, 10, 15 and 28 post dosing. There was a significant time effect for all tissues, while in most, the peak for α-tocopherol concentrations was observed at 3 d post dosing. Rate of uptake of vitamin E varied for different tissues, with liver, spleen and lung showing a pronounced uptake and muscle showing least.

Comparing the established biological potencies (IU/mg) of the various tocopherol sources to the estimate values for ruminants are as follows, respectively: d-α-tocopherol (1.49, 3.60); d-α-tocopheryl acetate (1.36, 2.58); dl-α-tocopherol (1.1, 1.37).

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

BIOAVAILABILITY OF VARIOUS VITAMIN E COMPOUNDS IN RUMINANTS

By

NICHOLAS HIDIROGLOU

August 1989

Chairman: Dr. L. R. McDowell
Major Department: Animal Science

This research was designed to investigate the bioavailability of various tocopherol sources for ruminants. In experiment I, d-α-tocopherol and dl-α-tocopherol suspended in sesame oil were administered intramuscularly in sheep at a level of 40 mg/kg body weight (b.w.). Sheep administered d-α-tocopherol and killed after 360 hr resulted in a higher ($P<.01$) bioavailability than for sheep injected with d-α-tocopherol or dl-α-tocopherol and sacrificed 240 hr after dosing. A more sustained increment in plasma α-tocopherol was observed with d-α-tocopherol than dl-α-tocopherol.

In experiment II, sheep were provided with 400 IU/d of either d-α-tocopherol, dl-α-tocopherol or their corresponding acetate forms for 28 d. Higher concentrations of α-tocopherol were observed in tissues of sheep fed d-α-tocopherol than the other tocopherol forms. In sheep fed d-α-tocopherol, the area under the plasma α-tocopherol time curve (AUC), was larger as compared to other forms of vitamin E supplementation.

Experiment III investigated the concentration of α-tocopherol in plasma and tissues of beef cows following a daily oral administration of 1000 IU of four tocopherol sources for 28 d. The d-α-tocopherol and its acetate ester increased plasma tocopherol concentrations faster (P<.05) than the racemic products, with the greatest response occurring with d-α-tocopherol. Tissue analyses confirmed that in adrenal gland, kidney, liver and lung, α-tocopherol concentrations were higher (P<.05) following d-α- than dl-α-tocopherol supplementation.

In experiment IV, dl-α-tocopherol and its ester were administered to sheep or cattle (100 or 50 mg/kg b.w., respectively) in a single oral dose. Blood plasma α-tocopherol tolerance curve area was higher (P<.05) in the dl-α-tocopherol group than in its ester form, as well as quicker (P<.05) time (h) for maximum plasma α-tocopherol concentration. A greater (P<.05) plasma tolerance curve area was observed in the cattle following administration of dl-α-tocopherol than its acetylated form.

In experiment V plasma and tissue vitamin E concentrations were determined following a 5 g dosage of dl-α-tocopherol intraperitoneally in sheep, which were slaughtered on d 3, 6, 10, 15 and 28 post dosing. There was a significant time effect for all tissues, while in most, the peak for α-tocopherol concentrations was observed at 3 d post dosing. Rate of uptake of vitamin E varied for different tissues, with liver, spleen and lung showing a pronounced uptake and muscle showing least.

Comparing the established biological potencies (IU/mg) of the various tocopherol sources to the estimate values for ruminants are as follows, respectively: d-α-tocopherol (1.49, 3.60); d-α-tocopheryl acetate (1.36, 2.58); dl-α-tocopherol (1.1, 1.37)

CHAPTER I
INTRODUCTION

Existence of an anti-sterility vitamin was brought to light in the early 1920s when evidence was obtained that female rats reared on a diet containing all the vitamins known at that time failed to reproduce, although they were apparently normal in other respects (Evans and Bishop, 1922; Mattill and Conklin, 1920; Sure, 1924). Although the rats would mate and conceive, pregnancy invariably was terminated by fetal death followed by resorption. The missing factor was characterized as a vitamin by Evans and Bishop (1922) and designated vitamin E. Evans et al. (1927), who found that the unsaponifiable fraction of wheat germ oil was a convenient raw material for chemical investigation, and isolated (1936) from wheat germ oil two alcohols, α- and β-tocopherol, both of which showed vitamin E activity.

The name tocopherol originated from the Greek tocos (childbirth), phero (to bear) and ol (alcohol). Two years later, its structure was elucidated (Fernholz, 1938), and shortly thereafter it was synthesized (Karrer et al., 1938). During the period 1930-1950, multiple varied deficiency disorders of animals were reported to be cured by vitamin E. Work by Schwarz and Foltz (1957) led to the identification of selenium as the factor other than vitamin E that could also prevent the degeneration of liver in rats. Dose of selenium required for complete protection against liver degeneration was about one five hundredth that of vitamin E. Biological potencies of various sources of vitamin E have been established primarily through the rat fetal resorption assay (Mason and Harris, 1947). From this assay the following biological potencies were established: 1 mg of dl-α-tocopheryl acetate = 1.0 IU; 1 mg dl-α-tocopherol = 1.1 IU; 1 mg d-α-tocopheryl acetate = 1.36 IU; 1 mg d-α-tocopherol = 1.49 IU. Recently, considerable effort has been made to determine the biological activity of various vitamin E compounds in species other than rats, and specifically humans (Horwitt et al., 1984; Baker et al., 1986).

In ruminants, although much is known about clinical signs of vitamin E deficiency (Rice and McMurray, 1982), very little attention has been given to the different potencies of various vitamin E compounds. This dissertation is particularly concerned with the biological activity of various vitamin E compounds in ruminants as well as the effect of mode of administration and its type of vehicle.

CHAPTER 2
REVIEW OF LITERATURE

Chemistry of Vitamin E

Structure

The term "vitamin E" today applies to a group of related chemical compounds, the tocopherols and tocotrienols. The structural formula of α-tocopherol is indicated in Figure 2.1.

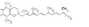

FIGURE 2.1. STRUCTURAL FORMULA OF α-TOCOPHEROL

The following tocopherols (Fig. 2.2) and tocotrienols (Fig. 2.3) are known:

Tocopherols	R_1	R_2	R_3	Chemical Name
α-Tocopherol	CH_3	CH_3	CH_3	5,7,8-Trimethyltocol
β-Tocopherol	CH_3	H	CH_3	5,8-Dimethyltocol
γ-Tocopherol	H	CH_3	CH_3	7,8-Dimethyltocol
δ-Tocopherol	H	H	CH_3	8-Methyltocol

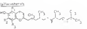

FIGURE 2.2 STRUCTURAL FORMULAS OF TOCOPHEROLS

Tocotrienols	R_1	R_2	R_3	Chemical Name
α-Tocotrienol	CH_3	CH_3	CH_3	5,7,8-Trimethyltocotrienol
β-Tocotrienol	CH_3	H	CH_3	5,8-Dimethyltocotrienol
γ-Tocotrienol	H	CH_3	CH_3	7 8-Dimethyltocotrienol
δ-Tocotrienol	H	H	CH_3	8-methyltocotrienol

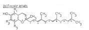

FIGURE 2.3 STRUCTURAL FORMULAS OF TOCOTRIENOLS

Figures 2.2 and 2.3 show that vitamin E exists as eight plant derived compounds that have a common 6-chromanol ring structure but that differ in the side chain and number of methyl groups. The four tocols have a phytyl side chain while the four trienols having trans double bonds at 3', 7' and 11' of the phytyl side chain. The methyl substituents occur in the ring at positions 5,7,8 (α-), 5,8 (β-), 7,8 (γ-), and 8 (δ-) in both the tocol and trienol series

The tocols contain three asymmetric carbons specifically at the 2 position (Fig. 2.4) in the ring and in the 4' and 8' position of the side chain, thus giving a total of eight possible optical isomers. The epimeric configuration at the 2 position is apparently dominant in determining biological activity.

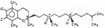

FIGURE 2.4: ASYMMETRIC CARBONS OF TOCOPHEROLS

The tocotrienols possess only one center of asymmetry at C_2 in addition to sites of geometrical isomerism at C_3' and C_7'. Thus number of stereo-isomers of the tocopherols and tocotrienols can exist. Natural α-tocopherol was shown conclusively to have the 2R, 4'R, 8'R configuration (Table 2.1). Thus, RRR-α-tocopherol can be used to denote the natural tocopherol isomer. The epimer of natural α-tocopherol, i.e., (2S, 4'R, 8'R)-α-tocopherol can be named 2-epi-α-tocopherol. A mixture of RRR and SRR-α-tocopherol can be obtained synthetically and is named 2-ambo-α-tocopherol (ambo = Latin for both). The reduction product of natural α-tocopherol is a mixture of four diastereo-isomeric α-tocopherols and can be called 4' ambo, 8' ambo-α-tocopherol. Synthetic α-tocopherol from synthetic phytol or isophytol is a mixture of four racemates in equal proportions and should be named all-rac-α-tocopherol.

Bieri and Prival (1967) reported that a group of synthetic tocopherol derivatives exhibited vitamin E activity. Among those reported were α-, β-, and γ-tocopheramines and N-methyl-β-, and N-methyl-γ-tocopheramines. The tocopheramine derivatives differ from the tocopherols in that an amine or an N-methyl amine group is substituted for the hydroxy group on the C-6 of the ring.

Synthesis

Chemical

Karrer et al. (1938) were the first to synthesize α-tocopherol by condensation of trimethylhydroquinone with phytyl bromide. Phytyl bromide was soon replaced by the natural phytol (Karrer and Isler, [illegible] Fieser et al., 1940) or isophytol (Karrer and Isler, 1941). All the tocopherols were subsequently synthesized and their structures elucidated.

TABLE 2.1. NOMENCLATURE FOR TOCOPHEROLS

Description	Configuration	Recommended	Other designation
Natural	2R,4'R,8'R	RRR α-	[d]-α-tocopherol, Steriochemically uniform product (only RRR-α)
Semisynthetic	2R,4'R,8'R	1 ambo α	dl-α-tocopherol. Two stereoisomers in the product (about 50% RRR-α historical standard)
Synthetic	Mixture of all	All rac-α	[dl]-α-tocopherol. Eight stereoisomers in the product (about 12.5% RRR-α)
Synthetic	2S,4'R,8'R	2-epi-α	[l]-α-tocopherol, Steriochemically uniform product (only SRR-α)

Two principal sources of vitamin E are in commercial use: d-α-tocopherol and esters (of acetate and succinate) of these compounds. The acetate esters are prepared chemically by reaction of the alcohol forms with acetic anhydride; they do not exist in nature. D-α-tocopherol is largely obtained from natural sources by molecular distillation. However, some of the d-α-tocopherol is prepared by further methylation of β-, γ-, and δ-tocopherols, or by hydrogenation of α-tocotrienol.

Synthetic dl-α-tocopherol and its esters are prepared from isophytol. This synthesis yields a mixture of eight isomers. Althoug semi-synthetic dl-α-tocopherol is racemic only at carbon 2 as prepared from natural phytol, samples of this compound may be available for research though it is not a commercial source of vitamin E.

Biosynthesis

Although conclusive proof of the biosynthetic route is still lacking, it is probable that the ring structure is formed from tyrosine by the pathway presented in Figure 2.5 (Whistance and Threlfall, 1967, 1968). Methylation of the ring structure by methyl transfer from methionine and formation of an initial isoprenoid side-chain from mevalonic acid completes the formation of a tocotrienol (Griffiths et al., 1968).

Properties of Vitamin E

Physical properties

Both d-α-tocopherol (RRR) and dl-α-tocopherol (all-rac) are practically insoluble in water but are almost completely soluble in oils, fats, acetone, alcohol, chloroform, ether, benzene, and other fat solvents. All tocopherols are stable to heat and alkali in the absence of oxygen and are unaffected by acids up to 100°C; they are slowly oxidized by atmospheric oxygen, a process which is increased rapidly by heat and catalyzed by ferric or silver salts. On exposure to light, the tocopherols gradually darken. They are not precipitated by digitonin. The commercial forms of vitamin E are d-α-tocopheryl acetate and dl-α-tocopheryl acetate.

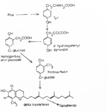

FIGURE 2.5. BIOSYNTHESIS OF THE RING STRUCTURE OF TOCOPHEROLS AND TOCOTRIENOLS

The melting point of RRR-α-tocopherol is 2.5 to 3.5°C; thus at room temperature, the compound is a viscous yellow oil which is soluble in aprotic solvents. Optical rotations of these tocopherols are very small and depend on the nature of the solvent. Table 2.2 gives the specific rotations of the spectra of natural tocopherols and tocotrienols in ethanol which show maxima in the range of 292-8 nm, infrared spectra show OH (2.8 to 3.0 μm) and CH (3.4 to 3.5 μm) stretching and a characteristic band at 8.6 μm.

TABLE 2.2 SPECIFIC OPTICAL ROTATIONS OF NATURAL TOCOPHEROLS

Compound	Solvent	$\alpha_{546.1}^{25°}$
α-Tocopherol	Ethanol Benzene	+0.32° -3.0°
β-Tocopherol	Ethanol Benzene	+2.9° +0.9°
γ-Tocopherol	Ethanol Benzene	+3.2° -2.4°
δ-Tocopherol	Ethanol Benzene	+3.4° +1.1°

Other properties include a molecular weight of 430.69; boiling point at 0.1 atm (used in molecular distillation) of 200 to 220°C, density 0.95 at 25°C in reference to water at 4°C, and a refractive index in sodium light spectrum at 20°C of 1.5045.

β-Tocopherol is a viscous, pale yellow oil, absorption maxima of 297 nm; $E_{1\,cm}^{1\%} = 86.4$

The absorption maxima for γ-tocopherol is 298 nm, $E^{1\%}_{1\,cm} = 92.8$; boiling point at 0.1 atm 210-210°C. Tocopheryl esters are more stable to oxygen but cannot function as anti-oxidants in this form.

Metabolic Degradation Products of Tocopherol

The chemistry of the oxidation of tocopherols is highly complex and has been reviewed by Kasparek (1980). Complete chemical degradation of the tocopherol molecule occurs upon treatment with chromic acid and potassium permanganate, while milder oxidation by nitric acid, silver nitrate, ferric chloride, auric chloride, ceric sulphate and nitrogen dioxide leads to formation of α-tocopheryl quinone (Fig. 2.6). With nitric acid and ferric chloride, α-tocored (Fig. 2.7) and α-tocopurple (Fig. 2.8) are also formed.

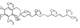

FIGURE 2.6. STRUCTURE OF TOCOPHEROL QUINONE

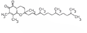

FIGURE 2.7. STRUCTURE OF α-TOCORED

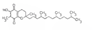

FIGURE 2.8. STRUCTURE OF α-TOCOPURPLE

Absorption and Storage of Vitamin E

Mechanism of absorption is similar to that of other fat soluble vitamins (Weber, 1983). Its absorption is closely associated with that of fat, and is accelerated by the presence of bile.

Excess tocopherol is eliminated in the feces. Simon et al (1956) observed that rabbits receiving a single dose of 10 to 15 mg 5-methyl-C^{14}-d-α-tocopherol succinate eliminated 65% of the dose via the feces in 3 days and 80% in 6 days, 90% of the radioactivity was identified as free α tocopherol by isotope dilution. It was concluded that the vitamin was re-secreted into the intestinal tract from the blood or via the bile. In confirmation by Krishnamurthy and Bieri (1963), who administered the vitamin orally, a second increase in fecal tocopherol was noticed which they did not attribute to coprophagy since the rats had tail cups. Mellors and McBarnes (1966) demonstrated that no significant amount of either tocopherol or its metabolites was introduced into the lumen of the rat guts via the bile or through secretion from mucosal cells. By using α-tocopherol labelled with radioactive C^{14}, Shantz (quoted by Harris and Ludwig, 1949) remarked that 80% of the vitamin E given in oil solution was excreted in the feces of rats.

Dju et al. (1950) observed that chickens receiving 1 g of α-tocopherol/day for a prolonged period eliminated 75% of the ingested quantity unchanged via the feces by 24 h. In contrast, chickens receiving a diet supplemented with 17.5 and 35 mg of α-tocopherol/kg only eliminated about 23% in the feces (Pudelkiewicz and Matterson, 1960).

Information on the absorption and excretion of tocopherols by farm animals is extremely sparse. In unpublished work with young calves, Blaxter and Brown (1952) indicated that about 25% of α-tocopherol added as the acetate to a diet of dried skimmed milk was excreted in the feces when the daily intake of the ester was from 25 to 100 mg. From such scanty data it is clearly impossible to draw the conclusion that species differ in their ability to absorb tocopherols, especially since great variation has been reported for a single species. Also, with labile substances like the tocopherols, a simple estimation of absorption based upon amounts present in the food and feces may be inaccurate.

These and other metabolic studies have shown that tocopherols are incompletely absorbed. The amount absorbed seems to depend on the requirement of the organism (Klatskin and Molander, 1952). Absorption and elimination also seem to depend on the amount in the diet.

There is a linear relation between the logarithms of ingested and liver tocopherol in rats (Bolliger and Bolliger Quaife, 1956). Griffiths (1960) noticed a linear relation between the logarithms of serum tocopherol and dietary concentration of vitamin E in chickens. The same author determined the ratio of pure α-, β-, γ- and δ-tocopherol in the livers of growing chicks as 100:41:19:0, but his findings are contradicted by Gray (1959), who found considerably lower tocopherol levels in rat plasma at high levels of tocopherol intake than Griffiths (1960) found in chicken serum.

Work of Dju et al, (1950) with hens included the observation that α-tocopherol was absorbed to a much greater extent than the γ- and δ-compounds. The serum tocopherol values of two hens which received weekly supplements of 1.6 g and 2.0 g α-tocopherol were 20.0 and 20.1 mg per 100 ml, but two hens that received weekly supplements of 0.8 and 1.0 g δ-tocopherol had serum tocopherol values of only 1.35 and 2.1 mg per 100 ml. Gamma-tocopherol appears to be only one-third to one-half as well absorbed as α-tocopherol in formula fed infants and supplemental vitamin E (1200 IU/day of all-rac-α-tocopherol: 800 mg α-tocopherol equivalents) in adults significantly reduced plasma γ-tocopherol levels, which suggested that intestinal uptake or plasma transport is more efficient for α- than γ-tocopherol (Jansson et al., 1981). Tocopherol esters are hydrolyzed prior to absorption, and both bile and pancreatic juice are necessary for absorption to proceed (Gallo-Torres, 1970). These facts support the idea that free tocopherol is absorbed by diffusion from a mixed micelle of fatty acids, monoacyl glycerols, bile salts and acids, cholesterol and other fat-soluble vitamins. Maximal absorption occurs at the junction of the upper and middle thirds of the small intestine (Hollander et al., 1975). After crossing the brush border into intestinal mucosal cells, tocopherol is not re-esterified but is incorporated as the alcohol into chylomicrons in mammals and enters the plasma via the lymphatic system (Behrens et al., 1982).

Desai et al. (1965) showed, in confirmation of studies by Weber et al. (1962) with rats, that l-α-tocopherol was absorbed as well as or better than the d-form of the vitamin. It appeared therefore that the differences in biopotency must be due to differences in retention whereby the d-epimer is retained much better than the l-epimer in the blood and perhaps in other tissues of the body. The results indicated the existence of an active carrier of d-α-tocopherol in the blood and tissues which has a greater affinity for the d-epimer than for l-α-tocopherol. Recently it has been reported that specific binding proteins exist for α-tocopherol in the cytosol and nuclei of rat liver tissue (Catignani et al., 1977; Guarnieri et al., 1980 Prasad et al., 1980) as well as in human erythrocytes (Kitabchi and Wimalasena, 1983) which are fairly specific for the natural stereoisomer. The exact nature of the binding of α-tocopherol in the cell is an elusive problem which needs investigation. Experiments by Desai and Scott (1965) and Scott (1965), comparing the oral administration of d- and l-α-tocopheryl acetates in the presence of graded levels of dietary selenium, indicated that selenium is involved in some unknown way in the retention of d-α-tocopherol in plasma. It remains to be determined if the differences in plasma levels of d- and l-epimers of α-tocopherol are the result of differences (1) in rate of excretion, (2) in rate of destruction, (3) in the affinity of the epimers for specific carriers, or (4) in chemical activity influenced by structural configurations.

Gallo-Torres (1970) reported the obligatory role of bile for the intestinal absorption of vitamin E into the lymph of rats. Only negligible amounts of radioactivity could be detected in the thoracic duct lymph when both bile and pancreatic juice were absent from the duodenum.

The lipid-bile micelle structure is required to transport the fat-soluble vitamin E across the "unstirred water layer" which represents the aqueous phase in the intestinal lumen immediately adjacent to the brush border of the microvilli. Vitamin E is absorbed, together with free fatty acids, monoglycerides, and other fat-soluble vitamins, by penetrating the epithelial cell through the apical plasma membrane of the absorptive cells in the brush border. Studies on absorption and transport of α tocopherol by Davies et al. (1971) showed that, for normal utilization of absorbed α-tocopherol, normal lipoprotein transport mechanisms are involved. Transfer of vitamin E from the absorptive cell thus requires several stages. In mammals, it must first pass through the lateral or basal plasma membrane of the cell, then through the basal lamina before entering the fluid of the lamina propria. From this location the vitamin enters the capillaries of the lymph and is transported in the chylomicrons (Dobbins, 1975). Apparently only small amounts of tocopherol are transported from the intestine via the portal vein in mammals, whereas all of the tocopherol absorption in birds occurs via the portal vein directly to the liver (Machlin, 1984).

The absorption of orally fed vitamin E follows the pattern of lipids in general and of fat soluble vitamins in particular (Desai et al., 1965, Wiss et al., 1962). The specific site of absorption is not well established. The small intestine is thought to be the major site of absorption for tocopherol even though some absorption takes place from the stomach of nonruminants and the rumen of ruminants (Blaxter and Brown, 1952; Roels, 1967). The presence of vitamin E in both the blood and lymph of animals suggests that absorbed vitamin E can be transferred by either the blood or lymphatic route (Roels, 1967).

Wiss et al. (1962) were also able to establish a mathematical relationship between the logarithms of tocopherol intake and plasma and liver concentration in chickens fed high doses of d-α-tocopheryl acetate (2,000 to 20,000 IU/kg of feed). Using [C^{14}]-dl-α-tocopheryl acetate, they demonstrated that maximal liver concentration was reached only after several hours, and persisted longer than the synthetic antioxidant ethoxyquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline), which attained a maximum within 30 min before declining rapidly. Most of the tocopherol was bound to the structural components of the liver cells, primarily mitochondria and microsomes.

Vitamin E is transported in the blood via lipoproteins. A rapid exchange among lipoprotein classes occurs with vitamin E after it enters the circulation via the chylomicrons. Since plasma tocopherol level is correlated with the total plasma lipid content, low density lipoprotein (LDL), the most common lipoprotein in human plasma, carries most of the plasma vitamin E. There is also a rapid exchange between tocopherol in the erythrocyte membrane and lipoproteins such that approximately 20% of the plasma tocopherol concentration is carried by red blood cells. Delivery to other tissue cells appears to be in association with the receptor-mediated uptake of LDL (Traber and Kayden, 1984).

Type and composition of diet influence absorption of vitamin E from the gut. Pudelkiewicz and Matterson (1960) reported that only about one-third of the d-α-tocopherol in alfalfa is available to chicks. The poor utilization is attributed to a fat-soluble compound in alfalfa that acts antagonistically to α-tocopherol decreasing its availability. An antagonistic relationship also exists between absorption of vitamin E and unsaturated fatty acids (Bunyan et al., 1968).

Generally, in animals, the uptake of vitamin E from the small intestine is lowered when tocopherol is fed in an oily form. The uptake is facilitated by bile salts (Simon et al., 1956).

When tocopheryl acetate ester was fed, most fecal tocopherol appeared as free tocopherol; however, a small portion appeared in the esterified form (Krishnamurthy and Bieri, 1963) indicating that hydrolysis of the ester took place in the digestive tract. Rosenkrantz et al. (1951) observed that approximately 8% of α-tocopherol given to human subjects was recovered as tocopherol quinone, suggesting the oxidation of tocopherol in the human digestive system.

No tocopherol or tocopheryl quinone was excreted in rat urine although large doses of vitamin E were administered (Kistakin and Molander, 1952). McArthur and Watson (1939) failed to detect any tocopherol in cow urine. Simon et al. (1956) reported that about 18% of tocopherol intake appeared in the urine of the rabbit and man after intravenous administration of isotopic vitamin E. Urinary radio activity was not attributed to α-tocopherol or tocopheryl quinone but to two polar metabolites identified as an acid and its lactone which were subsequently named tocopheronic acid and tocopheronolactone (Green et al., 1967). Work by Chow et al. (1967) confirmed the findings of Simon and associates.

Krishnamurthy and Bieri (1963) reported that only 0.5% of total activity was in the urine when the rat was sacrificed 24 h after oral administration of C^{14} α-tocopherol. In a long-term experiment, the same authors found that the activity in the urine was very low. Furthermore, no significant amount of vitamin E was lost in the form of CO_2.

Almost all studies on the pharmacological properties of vitamin E used doses between 200 and 800 mg/day. John Bieri, a leading American expert on vitamin E, had the following to say on dosage: "Nothing indicates that plasma levels that have been raised to 1.5 to 2 times the normal levels are less effective than even higher levels. These plasma levels, in subjects with normal absorption, can be achieved within 2 to 3 weeks of doses of 3 x 100 or 200 IU/day" (Bieri et al., 1983). This statement is aimed at discouraging extremely high doses of vitamin E.

The conditions of physiological absorption discourage very high individual doses of vitamin E. Absorbing dietary vitamin E usually presents few problems, since the quantities involved are small and the required mechanisms of fat digestion, which play an important role also in the absorption of vitamin E, are triggered automatically. Large individual doses of vitamin E, however, are not fully absorbed, two-thirds of them usually being excreted unchanged. Rate of absorption varies between 20 and 60%, and is dose dependent. Absorption of tocopherols depends on various factors, e.g. 1) pancreatic enzymes, 2) bile acids, 3) pH level of the intestinal contents, 4) intestinal motility, 5) other food components, in particular the fatty acid composition (Simon-Schnass et al., 1984).

Metabolism of Vitamin E

Although vitamin E is widely distributed among the tissues of animals and much is known about the clinical signs and symptoms of its deficiency, very little is known about the metabolism of vitamin E. The relative ease with which the tocopherols and their metabolites can be oxidized in vitro has complicated efforts to elucidate their mechanism of action (Martius and Furer, 1963).

Investigations of the metabolites of α-tocopherol in animal tissues were conducted by several workers with the anticipation that the nature of the metabolites would provide insight into the mechanism of action of vitamin E. Simon et al. (1956) isolated and characterized a metabolite of α-tocopherol from both rabbit and human urine. This oxidation product was given the common name tocopherol-5-methyl-C^{14} succinate. The same authors found that urine contained mainly metabolized tocopherol, while intact tocopherol accounted for the majority of the radioactivity in the feces. Mervyn and Morton (1959) reported the presence of α-tocopheryl quinone in a nephritic human kidney deficient in vitamin E. Alaupovic et al. (1961) found no evidence of α-tocopheryl quinone, α-tocopheryl hydroquinone, α-tocopheroxide, tocopheronic acid or tocopheronolactone in pig and rat livers. Csallany et al. (1962) identified two new metabolites of α-tocopherol isolated from the liver of the rat after injection of [5-methyl-^{14}C] d-α-tocopherol. About 25% of the recovered activity was unchanged α-tocopherol, about 19% was α-tocopheryl quinone, and about 50% was di-α-tocopherone, a dimer of oxidized -tocopherol. Csallany and Draper (1963, 1964) separated and identified the cis-and trans-isomers of dialpha-tocopherone. Draper et al. (1967) also identified a trimer of α-tocopherol in the mammalian liver. Krishnamurthy and Bieri (1963), and Plack and Bieri (1964) indicated that oral and intraperitoneal administration of ^{14}C α-tocopherol to rats and chicks gave rise mostly to unchanged tocopherol in the tissues of the tested animals. Mellors and McBarnes (1966) confirmed the above findings. Following intravenous injection of ^{3}H-α-tocopherol, Shiratori (1974) observed large amounts of radioactivity in dermal tissue suggesting that skin may play a role for tocopherol secretion or excretion.

Vitamin E Deficiency

The absence of vitamin E in the diets of animals poses health problems and affects various functions.

Effects of Deficiency on the Reproductive System

In 1920, Mattill and Conklin showed that a deficiency in vitamin E led to reproductive disorders and lesions of the genital apparatus in rats. The data were confirmed in 1922 by Evans and Bishop. As a result of some premature generalization of these observations, vitamin E came to be considered as an anti-sterility factor and its therapeutic use was widely advocated. Now, however, it is apparent that the role of this factor varies widely among species.

In males

Rodents. In rats, vitamin E deficiency results in testicle degeneration (Mason, 1954). The gland is reduced to half its normal size and becomes brownish and flaccid. Degenerative lesions affect the spermiduct and lead to the complete cessation of sperm production. Neither Sertoli cells nor the interstitial gland appear to be affected. Some changes in related glands (seminal vesicles and prostate) have been reported.

It appears that guinea pigs (Curto, 1966), hamsters and rabbits are also susceptible, although less markedly so. In rabbits, in particular, testicle lesions seem minor when compared to the

widespread effects of vitamin E deficiency on [illegible]
Harris, 1947).

Other species. Monkeys and roosters are also susceptible to vitamin E deficiency. Stallions, bulls, rams, billy goats, and perhaps male mice, are not (Baxter and Brown, 1952).

In females

Rodents. In rats, vitamin E deficiency does not appear to affect the development and functioning of the genital systems (estrus, ovulation, fecundation and nesting), but it has a major impact on prenatal development, mainly during the days following nesting. The uterus and the ovaries become covered with brownish yellow pigment while the basic signs are early embryo mortality followed by resorption as a result of placental lesions which may sometimes hemorrhage. When there is a chronic deficiency, the rat may produce only one or two live young, while the others die (Martin and Moore, 1939). The survivors present serious muscular dystrophy lesions (Bonetti and Stirpe, 1963). This cause should be kept in mind when a reduction in fecundity in this species occurs.

Other species. Where there is vitamin E deficiency, feto-placental hemorrhages and some degree of embryo mortality have occasionally been noted in sows and rats. Surviving piglets develop poorly and present muscular dystrophy. It may be concluded that sows are susceptible to vitamin E deficiency, though to less extent than rats (Lamesk, 1962).

The effects on reproduction in cows, ewes, goats and mares are nil, although these species are very susceptible to muscular dystrophy lesions, especially in the very young (Gullickson, 1949). However, a high placental susceptibility to vitamin E deficiency as noted in rats and sows, apparently in mice and perhaps in rabbits, also appears to exist in cows according to Trinder et al. (1969). These authors noted a high frequency of placenta retentions (19%) in non-brucellic cattle in areas where muscular dystrophy in lambs was widespread. Intramuscular injections of vitamin E (680 IU) and potassium selenate (15 mg) one month before parturition markedly reduced the frequency of retention. Administrations of potassium selenate only were less effective, and earlier injections (7 weeks prepartum) ineffective.

A Variety of Disorders Due to Vitamin E Deficiency in Domestic Animals

Vitamin E deficiency causes various disorders in most animal species. An exhaustive description would be tedious and time consuming. Observations will be limited to three frequently affected species: chickens, lambs and calves.

Chickens

Encephalomalacia. Clinical signs of nutrition-related encephalomalacia are exclusively nerve-related (Dam, 1944). The first manifestation is ataxia. Next, there may be clonic contractions and especially trembling. The chicken keeps its head tucked under, as if rolled into a ball; then paralysis sets in and it falls down in lateral decubitus. Death is the usual outcome of this clinical form which entails 20 to 80% losses, depending on the intensity of the deficiency and the susceptibility of the subjects. Lesions are significant because they make it possible to distinguish this disorder from another neural disease of viral origin in chickens, infectious encephalomyelitis

Characteristics of nutrition-related encephalomalacia (Adamstone, 1947) are (1) discrete hepatic lesions; the liver is often pale, degenerated, speckled with sanguineous suffusions; (2) intestinal hemorrhagic lesions, occurring mainly around the duodenum and involving the muscularis; and (3) cerebral lesions, more specific, macroscopically detectable in some cases, but always visible upon histological examination.

Upon opening the cranium, the cerebellum is edematized, covered with hemorrhagic patches, and of very poor consistency. Microscopic examination confirms the edema and hemorrhages, and in addition makes it possible to detect changes in Purkinje cells, which appear retracted, with granulous cytoplasm and pycnic nuclei.

In viral encephalomyelitis, the nerve lesions are different from the encephalomalacia with perivascular lymphocytic infiltration. In addition, lymphocytic infiltration can be detected in the pancreas, the succenturiate ventricle and the muscularis of the gizzard.

The distinction between these two diseases is an important one, since in encephalomalacia the responsible agent is the feed, whereas in encephalomyelitis it is the breeder, since contamination of the chickens occurs in ovo.

Exudative diathesis Exudative diathesis appears in chickens at the same age as does encephalomalcia and is due to a vitamin E deficiency associated with a deficiency in sulfur amino acids (Bunyan et al., 1962). It is characterized by the appearance of subcutaneous edema which deforms the head and neck and gives the feet a greenish tint, hence its other name of "green foot disease" (Dam and Glavind, 1939).

Lesions are also edematous and affect the entire subcutaneous connective tissue. This tissue has a gelatinous aspect and presents hemorrhagic spots, in particular on major muscle masses and in the periarticular area. In addition, whitish muscular dystrophy patches are found fairly regularly in the gizzard area.

Muscular dystrophy Muscular dystrophy appears around the age of one month. It takes the form of locomotor troubles and weight loss and entails considerable mortality. Lesions affect striated muscle fibers which present zenkerism. They are closely comparable to those of myopathy-dyspnea in calves and muscular dystrophy in lambs. Muscular dystrophy in chickens appears to be due to a deficiency of both vitamin E and selenium.

Lambs

This deficiency is also associated with clinical manifestations appearing in areas where the soil is selenium-poor (Muth et al., 1961). Winter lambs born of ewes which are more or less underfed pay the heaviest consequence, particularly where ewes have two or three young, whose requirements cannot easily be met (Oldfield et al., 1963).

Clinical signs begin inconspicuously with listlessness, sick lambs dislike moving about, and have trouble following the rest of the flock. However, the appetite remains normal. Gradually, the gait changes; steps become smaller, posteriors are stiff and splayed, and are affected with trembling and very painful to pressure. Standing becomes increasingly difficult and then impossible, with animals dying in a few days from heart failure or malnutrition. Death is preceded by blindness in most cases. Lesions affect the large muscle masses and the most active muscles. They are found mainly on psoas, crural and also on intercostals and myocardium.

Affected areas are discolored, whitish, and have the appearance of fish flesh. Extent of discoloration is varied; sometimes an entire muscle group may be affected, sometimes only a few fibers--this gives the muscle a striated, cashed effect. A histological examination of the lesions shows that the striated muscle fibers harbor zenkerism.

Calves

Vitamin E deficiency is also accompanied by selenium deficiency, which is characterized by white muscle disease (Blaxter and Sharman, 1953). It usually manifests itself in calves which are fed on milk only and is associated with rapid-growing animals with high nutritional requirements. Clinical signs appear between 15 days and 3 months, either in stables or at the time of pasturing. In stables, clinical signs appear gradually. At first, the gait stiffens; animals are stooped and move slowly when going to suck. Stiffness increases and standing becomes impossible; polypnea develops.

The polypnea is the only respiratory manifestation of this syndrome. It is accompanied by heart disorders which are characterized by a pendulum rhythm and a reduction in heart sounds upon auscultation, the characteristic signs of myocarditis. Finally, calves remain in lateral decubitus, with swollen, painful muscles affected by trembling, and die of either pulmonary edema or heart failure.

In pasture calves, the syndrome appears when they are released in the spring. They begin by running and jumping, but suddenly one or two may slow down and stand still, with a stiff, awkward stance. Then they lie down and are unable to get up. In this case also, heart and respiratory disorders lead to swift death (King and Maplesden, 1960).

Vitamin E-selenium lesions are identical with those of lambs, both macro- and microscopically. From the foregoing it will appear that vitamin E deficiency appears to lead to two major types of syndromes: (1) degenerative muscular lesions, common to a number of species and generally related to selenium deficiency, and (2) vascular lesions (edema and hemorrhages), well known in poultry, affecting mainly nervous and subcutaneous connective tissues.

Biological Activity of Vitamin E

Biological activities of the various tocopherols are different. Extensive and very complex studies on several experimental animal species have been conducted to determine the biological activity of vitamin E components which have been described in the literature for many years.

Methods of Assay

Determination of biologically active vitamin E is complicated by many interrelated factors. Chemical analyses must contend with the fact that eight different tocopherols occur in nature, and synthetic forms are supplemented in many foods and feeds as dl-α-tocopherol or as d- and dl-α-tocopheryl acetate or succinates. Bioassays may be confounded by the levels of antioxidants, prooxidants, selenium, and other factors which may alter the biological activity of the vitamin.

The biological activity of tocopherols (and of substances with vitamin E-like activity) can only be assessed with a bioassay technique which determines the ability of the tocopherol to reverse clinical signs of vitamin E deficiency. The manifestations of a deficiency differ markedly in different species. The signs of deficiency most commonly used in bioassay work are rat sterility (fetal resorption in the female and testicular atrophy in the male), rat erythrocyte hemolysis, and muscular dystrophy in a number of different species. A comprehensive review of bioassay methods for tocopherols has been reported by Bliss and Gyorgy (1967) and Ames (1971). Other tests, such as the liver storage, and elevation of plasma tocopherol, have also been used. These latter methods are not direct measures of biological activity in vivo, but they do reflect the relative absorption of the test compounds as well as their turnover in the liver or red blood cells. In general, the erythrocyte hemolysis and tissue storage tests correlate well with the in vivo procedures and may be correlated with alterations in red blood cell half-life observed in vitamin E-deficient humans. Therefore, although caution should be used in interpreting results, these methods are convenient and may be useful.

Resorption sterility test

The fetal resorption test in female rats is the classical means of conducting the bioassay of tocopherols (Mason and Harris, 1947, Ames et al., 1963). It measures the all-or-none response to the test substance by successfully mated vitamin E-deficient female rats in producing viable offspring.

The resorption sterility test in rats is frequently used in addition to the hemolysis test. This test system was used to determine the biological activity of the eight enantiomers of the synthetic all-rac-α-tocopheryl acetate (Weiser and Vecchi, 1982).

The resorption sterility test is based on the fact that female rats supplied with a vitamin E-deficient diet are unable to carry their offspring to term. The fetuses die before the end of the gestation period and are subject to intra-uterine resorption. Weaned rats are given a vitamin E-free diet for 3 to 4 months at which time their sterility is assessed by test matings with fertile males, insemination is checked by vaginal smear for spermatozoa. Different levels of standard RRR-α-tocopherol and the unknown substances are added to olive oil (or sometimes to portions of diet) which is administered to pregnant females through the 5th to 9th days of pregnancy. If dosing is delayed beyond the 10th to 12th day of pregnancy, vitamin E is ineffective. The animals are killed on the 19th day, i.e., 2 days before parturition, and examined for living fetuses and for sites of implantation of fetuses that have been resorbed. Animals having less than four implantation sites are discarded; and those having one or more living fetuses are regarded as positives, while rats with no live young are negative. The vitamin E activity of the test substance is determined by calculating the percentage of rats in each test group that showed a positive response and by plotting the calculated probits against dose (or log dose) to derive the 50% fertility dose. The ratio of the 50% fertility dose of the standard to the unknown substance is the relative vitamin E biopotency of the test substance. It will be clear from the foregoing that the fetal resorption test is tedious and time consuming and that meaningful results can be achieved only by carrying out a large number of tests on any given test substance, but it must still be regarded as the final reference point in assessing vitamin E biopotency of an unknown substance.

This test model is species specific in its validity The test results cannot be directly applied to man, but because this model can be standardized it can be used for comparisons of structure and activity relationships. As in any other specific animal model, its validity limits must be taken into account. The results obtained for relative activity of the free, easily oxidizable tocopherols vary greatly in this model from one team of investigators to another (different laboratories) since the free tocopherols are easily inactivated by other components in the diet of the experimental animals. Knowledge of this interference dates back to the 1940s, and consequently this model is used mainly to test and compare acetylated tocopherols.

The results tabulated in Table 2-3 show that it is the configuration in the phytol side chain which decisively affects the activity in the biological system. The 2R, 4'R, 8'R configuration, which is identical to the naturally occurring form, has the highest activity (100% relative biological activity). The configuration in the C-2 position is particularly important. It is noted that in all the diastereomer pairs the 2S form has less activity than the 2R form. The greatest difference in activity is noted in a comparison between the 2S, 4'R, 8'R-α-tocopheryl acetate and the 2R, 4'R,8'R-α-tocopheryl acetate, which is identical to the naturally occurring form. The relative activity is only 21 to 31% for the SRR form. The SSS isomer exhibits the surprisingly high relative activity of 60% compared to the RRR isomer.

Observations on the biological activity of stereoisomer mixtures vary. Ames (1979) showed on the basis of 19 tests that the activity of all-rac-α-tocopheryl acetate does not exceed the calculable activity. By contrast, Weiser and Vechi (1982) found in two experiments that stereoisomer mixtures had a synergistic effect in the rat resorption sterility test.

Erythrocyte hemolysis test

This test is based on the protection of erythrocytes by vitamin E against hemolysis induced by dialuric acid or hydrogen peroxide. Rats weighing about 100 g are fed a diet free of vitamin E for a depletion period of 3 to 4 weeks. A sample of blood is then taken from each rat and tested for hemolysis induction by addition of dialuric acid. Only animals showing a degree of hemolysis of 96 to 99% are retained in the test. In a preliminary trial with five animals per dose, dosages of vitamin E are determined which will reduce the hemolysis to a range of

TABLE 2.3 RELATIVE ACTIVITY OF VARIOUS α-TOCOPHEROL STEREOISOMERS AS DETERMINED BY RESORPTION STERILITY TEST IN FEMALE RATS

Standard with 100% biological activity: RRR-α-tocopherol acetate α-tocopherol acetate isomer	Relative activity in relation to the standard (in percent)
2R,4'R,8'R	100
2R,4'R,8'S	90
2R,4'S,8'S	73
2S,4'S,8'S	60
2R,4'S,8'R	57
2S,4'R,8'R	31
2S,4'R,8'S	37
2S 4'S,8'R	21
all-rac-α	32
(8 enantiomers)	67–77
2-ambo-α	60.2
(2 enantiomers)	73

approximately 20 to 80% for both the standard and the test preparations For the bioassay, two or three doses are selected from within this range for the standard and for each sample to be assayed. Each dose is dissolved in 0.2 ml olive oil and administered orally by stomach tube. After 40 to 44 hr the percent erythrocyte hemolysis is again measured The use of dialuric acid as a hemolytic agent for erythrocytes derived from vitamin E-deficient animals was introduced by Rose and Gyorgy (1952), and later improved by Friedman et al (1958)

As an alternative to dialuric acid, hydrogen peroxide may be used as the hemolytic agent (Gyorgy et al., 1952). it should be noted that there is no spontaneous hemolysis *in vivo* in vitamin E-deficient animals. However, the rat hemolysis test is not a direct measure *in vivo* of the biopotency of a vitamin E-active substance, since the test is carried out on blood derived from rats given a vitamin E-deficient diet. Furthermore, the results obtained need to be interpreted with great caution since the period of giving the deficient diet needs only be quite short before a positive test can be obtained, and the animal would not necessarily show overt signs of vitamin E deficiency such as sterility The test is thus regarded by many as a measurement of the vitamin E content of the blood rather than as an assessment of the vitamin E status of the animal. With this difficulty in mind, however, the test provides a means of assessing the probable vitamin E activity of an unknown substance and has been found to agree well with the results obtained by fetal resorption (Harris and Ludwig, 1949) and chick liver storage (Pudelkiewicz et al , 1960).

Of all the test systems, the erythrocyte hemolysis test (EHT) is of special importance. This test can be done not only as a pure *in vitro* variant but also as an *in vivo-in vitro* form. The EHT provides a means of indirect measurement of the human vitamin E status and correlates well with the tocopherol concentrations in blood plasma. Individuals with decreased tocopherol concentration are characterized by an increased hydrogen peroxide-induced sensitivity of the erythrocytes to hemolysis in the blood serum.

The most commonly used hemolytic agent is hydrogen peroxide. Washed erythrocytes are incubated in a hydrogen peroxide solution (about 2%) over a period of 3 hr. Hemolysis liberates hemoglobin during incubation, correction for this hemoglobin is made by determining the amount of hemoglobin after incubation with distilled water which corresponds to 100% hemolysis (without addition). The hemolysis result obtained with hydrogen peroxide is given as a percentage.

The relative activity of d-α-tocopherol and d-γ-tocopherol in the *in vitro* EHT is tabulated in Table 2-4 (Brubacher and Weiser, 1968). Erythrocytes from rats which were given a vitamin E-deficient diet were incubated in a test tube together with the various tocopherols and then hemolyzed with dialuric acid. The tocopherol esterified with acetic acid is inactive in this test. D-α-tocopherol has again been shown to be biologically superior to d-γ-tocopherol.

The hemolysis test was originally conceived as an *in vitro* test but was later modified. This modified test differs from the above-mentioned *in vitro* test in that the vitamin E active substance is

TABLE 2.4. RELATIVE BIOLOGICAL ACTIVITY OF VITAMIN E COMPOUNDS IN THE IN VITRO HEMOLYSIS TEST; EXCERPT FROM BRUBACHER AND WEISER (1968).

Compound	Relative activity (%)
d-α tocopherol	100
d-γ tocopherol	30

administered orally to the experimental animals 2 days before blood samples are taken, after which the hemolysis test is undertaken. This test model shows that even the forms which are esterified with acetic acid exhibit vitamin E activity after they have been liberated by enzymes in the intestine (Table 2.5). Here again it is the d-α configuration, both in the free form and in the form esterified with

TABLE 2.5. RELATIVE BIOLOGICAL ACTIVITY OF TOCOPHEROLS AND THEIR ESTERS WITH ACETIC ACID IN THE IN VITRO/IN VIVO HEMOLYSIS TEST IN RATS; EXCERPT FROM BLISS AND GYORGY (1969).

Compound	Relative activity (%)
3 d-α-tocopherol	132.8
4 D-α-tocopheryl acetate	147.4
5 all-rac-α-tocopheryl acetate	100.0 (standard)
6 D-γ-tocopherol	21.7
7 D-γ-tocopheryl acetate	18.9

acetic acid, that possesses the highest biological activity. Although the test method is not precise enough to differentiate d-α-tocopherol from its acetate, the two forms with the natural configuration are nevertheless significantly superior to the synthetic all-rac-α-tocopheryl acetate.

Plasma and erythrocyte concentration test

Vitamin E acts as an antioxidant in biological membranes (Dam, 1957). Therapy is designed to achieve a high tocopherol concentration in these membranes.

In test rats which were reared on a vitamin E-deficient diet, free d-α-tocopherol was compared with synthetic all-rac-α-tocopheryl acetate to determine their affect in producing changes in vitamin E concentration in plasma and in erythrocytes after intravenous or oral administration (Ogihara et al., 1985). The tocopherol concentration in the red blood cells was investigated as an indicator of the physiologically available tocopherol in biological membranes.

Although intravenous administration of all-rac-α-tocopheryl acetate produced a higher tocopherol level in plasma than RRR-α-tocopherol 6 h after infusion, the concentration in the erythrocytes at the same time increased by 100% after infusion of RRR-α-tocopherol compared to the response when all-rac-α-tocopheryl acetate was infused.

Differences between the natural, free vitamin E and the synthetic acetate ester were even more pronounced in these test animals after oral administration. Plasma concentration 6 h after oral administration of d-α-tocopherol was 40% higher than after all-rac-α-tocopheryl acetate intake. Uptake in the erythrocytes (h after oral administration of d-α-tocopherol was 280% higher than after administration of the synthetic material. Direct comparison at 24 and 48 h after oral administration indicated that uptakes of the free d-α-tocopherol in the erythrocytes was 300 and 255% greater than uptake of the ester. These test results provided impressive support for the superiority of the natural vitamin E structure as previously shown in clinical studies.

Liver storage tests

Liver storage tests are based on the observation that amount of tocopherol stored in the liver of rats and chicks has a linear relationship to the level of vitamin E consumed

Pudelkiewicz et al. (1960) used day-old White Plymouth Rock male chicks fed a vitamin E-deficient diet for 13 days for assay of liver storage of vitamin E. Smallest and largest chicks are discarded, with those remaining distributed by weight into groups of eight chicks each. The tocopherol standards and the unknown materials to be assayed are then mixed with the basal diet and the chicks in all lots are pair-fed to the lot eating the least amount of feed over a period of 14 days. Tocopherol content of pooled livers from each group was then determined by chemical assay using the Emmerie-Engel reaction after careful extraction, molecular distillation, and chromatographic separation of the tocopherols.

Muscular degeneration tests

Muscular dystrophy has been used as a means of measuring vitamin E biopotency in rabbits (Hove and Harris, 1947; Fitch and Diehl, 1965) and chicks (Scott and Desai, 1964, Desai and Scott, 1965).

In chickens, muscular degeneration is evaluated directly after 3 to 4 weeks on deficient or supplemented diets. The breast muscle is examined and scored from 0 to 4, depending on the severity of the lesion. Indirect measure of muscular degeneration, such as creatinuria and levels of plasma aspartate aminotransferase and lactic dehydrogenase, have also been used in ducks and rabbits. The time of onset of creatinuria or the extent of muscular dystrophy, assessed on a scale, are both used as biological measures, and the effect on these parameters of standard RRR-α-tocopherol or a test material is assessed as the basis of the assay. A method based on the reversal of muscular degeneration in the rat, using plasma pyruvate kinase levels as an index, appears to be quite reliable, rapid, and sensitive (Machlin et al., 1982).

D-α-Tocopherol Equivalents

A basis for establishing the biological activity of vitamin E preparations is necessary to ensure uniformity of dosage. In 1978 the International Union of Nutritional Sciences (IUNS), therefore, recommended that d-α-tocopherol be used as the standard compound and that the biological activity be specified in activity equivalents (Fromming, 1984).

D-α-tocopheryl acetate can be easily obtained in chemically and isomerically pure form. The relative biological activities of the tocopherols have been determined in experimental animals and are now internationally accepted. They are calculated as follows:

1 d-α-tocopherol equivalent (mg)

= d-α-tocopherol x 1

= d-α-tocopheryl acetate x 0.91

= all-rac-α-tocopheryl acetate x 0.67

= d-γ-tocopherol x 0.1

These equivalence values have been disputed (Ames, 1979, Leth and Sandergaard, 1983), however, in 1983 they were confirmed by a WHO/FAO Expert Committee (WHO, 1983). Evidently the experts did not consider it necessary to change the relative biological activities of tocopherols based on animal experiments. More recent human studies suggest that the findings on which the conversion factors are based, i.e., experiments with rats given a vitamin E-deficient diet, should be regarded as species-specific for the experimental animals.

A comparison of the tocopherol concentrations in human tissues and cells emphasizes the physiological significance of free RRR-α-tocopherol relative to other tocopherols and derivatives. The vitamin E requirement in man is satisfied by the preferential utilization of d-α-tocopherol in the diet. That d-α-tocopherol possesses the relatively greatest biological activity has been confirmed by a multitude of relevant in vitro and in vivo tests and particularly by clinical studies. Accordingly, RRR-α-tocopherol is the actual vitamin E in man.

The United States Pharmacopeia (USP) (1980) in Table 2.6 shows the recommended weight/unit relationships for six tocopherols.

TABLE 2.6 USP WEIGHT/UNIT RELATIONSHIPS OF TOCOPHEROL.

1 mg all-rac-α-tocopherol = 1.1 units
1 mg all-rac-α-tocopheryl acetate = 1 unit
1 mg all-rac-α-tocopheryl succinate = 0.89 unit
1 mg RRR-α-tocopherol = 1.49 units
1 mg RRR-α-tocopheryl acetate = 1.36 units
1 mg RRR-α-tocopheryl succinate = 1.21 units

Role of Vitamin E in the Respiratory Chain

It has long been known that the hepatic necrosis that accompanies vitamin E deficiency is preceded by a reduction of cell respiration shown on cross sections of prenecrotic liver (Chernick et al., 1955). Vitamin E supplementation immediately reestablishes respiration, both *in vivo* and *in vitro* (Schwarz, 1955). Selenium is also effective, but only *in vivo* which seems to indicate that a different mechanism is at work (Schwarz, 1972).

Cell respiration takes place at the level of the mitochondria, through the respiratory chain, which consists of a series of hydrogen-transport systems which intervene in an order determined by their oxidoreduction potentials. Energy released by this hydrogen transport system (which corresponds to oxidation of the substrate) is partially stored in the form of adenosine triphosphate (ATP). Determination of the P/O ratio (number of atoms of inorganic P which disappear when one atom of oxygen is reduced), which is generally equal to 3, provides a measure of cellular respiratory activity.

One of the links in the respiratory chain is ubiquinone (or coenzyme Q) which intervenes between the flavin-nucleotide and cytochrome system. The structure of ubiquinone is similar to that of α-tocoquinone, a metabolite of α-tocopherol which, along with the corresponding hydroquinone can form a hydrogen transport system comparable to that of ubiquinone.

In the anemias observed when vitamin E deficiencies occur in primates, an improvement is obtained upon administration of ubiquinone as with vitamin E, thereby indicating the similarity of action for the two substances.

It is likely that vitamin E intervenes in the respiratory chain, and thereby contributes to the metabolic equilibrium of cells (Schwarz, 1972).

Intervention of Vitamin E in Hematopoiesis

Anemia, which accompanies vitamin E deficiency in primates, and the decoloration of the muscles in muscular dystrophy in many species (resulting from rarefaction of the myoglobin) have led to research into the possible role of this vitamin in heme synthesis.

Heme synthesis results from a series of transformations starting with glycine and succinyl-CoA, leading to the formation of δaminolevulinic acid (ALA), followed by the condensation of two molecules of ALA to form porphobilinogen. These two transformations are catalyzed by two enzymes, ALA-synthetase and ALA-dehydratase, respectively.

Nair et al. (1970) have shown that these two enzymes are under the control of vitamin E. The fact that actinomycin D (which inhibits transcription from DNA to RNA) impeded the ability of vitamin E to reestablish heme formation in the mitochondria of deficient tissues suggest that vitamin E might intervene at this level by acting as a specific repressor of the transcription of the enzymes of ALA (Nair, 1972).

Since heme is not only a constituent of hemoglobin, but also of myoglobin, cytochromes and various oxidoreduction enzymes, it is possible to better understand certain aspects of vitamin E deficiency, namely the decoloration of the muscles in muscular dystrophy due to rarefaction of the myoglobin. Similarly, the decoloration which various researchers have observed in the mitochondria of deficient tissues reflects a rarefaction of the cytochromes, which contributes to a lowering of cell respiration by disruption of the respiratory chain at the level of the cytochromes.

Biological Role of Vitamin E

Antioxidant Activity

It has been known for some time that vitamin E is an excellent fat-soluble antioxidant (Dam, 1957; Tappel, 1962) although the physiological significance of this trait is only now becoming clear.

Because of its antioxidant effect, vitamin E is able to entrap free radicals before they can attack life-supporting cell structures. Free radicals are, by definition, chemical compounds which contain an odd number of electrons. Free radicals can be formed in biological systems through homolytic cleavage of covalently bonded organic compounds resulting in bond splitting, whereby each fragment retains one electron of the original bonding pair

$$R - \overset{\overset{H}{|}}{C} = C,$$

Bond splitting as such always gives rise to a pair of radicals. Radicals can also be formed from a capture of an electron by a molecule as illustrated below:

$$O_2 + e^- \rightarrow O_2^{-}.$$

The formation of superoxide anion radical results in a net negative charge because of the extra electron in its possession. Capturement of electrons by molecular O_2 is known to occur by its interaction with flavin oxidoreductase systems (Fridovich, 1976). For a better understanding of these phenomena, the effect of free radicals upon the breakdown of an essential fatty acid as described by Leibovitz and Siegel (1980) is presented in Fig. 2.9.

In a fatty acid, at a carbon atom adjoining a double bond, an agressive substance (aS) removes hydrogen (I), resulting in the formation of a fatty acid radical (II). This radical reacts with oxygen to form a peroxy fatty acid radical (III). This new radical in its turn removes hydrogen from another essential fatty acid, resulting in the formation of a hydroperoxy fatty acid (IV) and again in a fatty

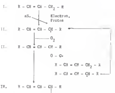

FIGURE 2.9. SCHEMATIC REPRESENTATION OF THE PEROXIDATION OF UNSATURATED FATTY ACIDS

acid radical which can react further. Once this chain reaction has started, it continues until all unsaturated compounds have been broken up, self-quenching occurs, or the radical is entrapped by an antioxidant (Chow, 1979).

Autoxidation eventually results in some form of degradation of the material affected. For example, foodstuffs such as corn oil, butter and margarine become rancid. The process is generally represented by four elementary reactions:

Initiation: production of $R\cdot$ (1)

Propagation: $R\cdot + O_2$ ----- $ROO\cdot$ (2)

$ROO\cdot + RH$ ----- $ROOH + R\cdot$ (3)

Termination: $ROO\cdot + ROO\cdot$ ----- Molecular products (4)

In this scheme, RH represents a lipid molecule and R. the carbon-centered radical derived from it by removal of a hydrogen atom. (Propagation can also occur by addition of the peroxyl radical to a carbon-carbon double bond.) These reactions can be prevented by antioxidants (Lundberg, 1962).

$ROO\cdot + XH_2$ ----- $ROOH + XH\cdot$

$XH\cdot + XH\cdot$ ----- $X + XXH_2$

XH = antioxidant

At the cellular level, autoxidation is clearly undesirable because it threatens the integrity, protective and organizational properties of the membranes which enclose cells and organelles, thus placing the survival of the cell itself in jeopardy.

In the last few years much research has been done concerning free radicals and into how various substances act to inhibit them (Cheeseman et al., 1984).

Free radicals are involved in two types of biological phenomena. Some appear in normal cell reactions, others in abnormal reactions and in processes associated with the aging of organisms. All biological oxidations do not necessarily give rise to such radicals. The mechanisms by which they are produced are numerous as they result from many types of reactions. These reactions include the peroxidation of lipids, in the course of which, under certain conditions increase depending upon the rate of formation of free radicals. It has also been shown that some processes, whether enzymatic or not, produce reactions of the same type when some of the cell constituents released are exposed to the direct action of oxygen in a free environment or in the absence of inhibiting agents. Some researchers, such as Pryor (1970), do not rule out the possibility that radicals may escape an electron transport chain, than causing rapid and destructive oxidations.

It may seem paradoxical, as Pryor (1971) points out, that oxygen, which cells need to release energy from food, is so reactive that it manages to trigger, alongside the usual processes, some undesirable parallel reactions oriented toward actions detrimental to the phenomena essential to life. In the course of such oxidative reactions, it causes damage to the cells by producing unfavorable modifications in the structure of their membranes, in the membranes of the intracellular organelles and in the endoplasmic reticulum.

For its part, ozone, a layer of which shelters the planet from short-wavelength ultraviolet radiation and known to be harmful, also causes oxidations which are detrimental to biological cell systems.

Study of the chemical composition of the cell membranes shows that they are formed by an association of lipids and proteins performing important functions such as electronic phenomena, active transport, glycolysis, biosynthesis of lipids and proteins, etc. Lipids are the most important constituent not only of these cell membranes, but also those of the intra-cellular organelles including the the mitochondria and microsomes. Many factors suggest that the membranes consist of a file of lipids, rich in triglycerides and phospholipids, between 40 and 65 A in thickness, covered on both sides by a layer of proteins each about 10 A thick (Lehninger, 1975). The peroxidation of the lipid constituents of the membranes, which contain a high proportion of mono and polyunsaturated fatty acids (C16:1; C18:1; C18:2; C18:3; and C20:4) disturb their metabolic functions (Tappel, 1972). The polyunsaturated fatty acid moieties of lipids are characterized by the presence of two or more carbon-carbon double bonds which are separated by a methylene-CH_2-unit. Methylene units are very susceptible to attack by radicals as peroxyl and ROO· with an extremely rapid combination with oxygen to form peroxyl radicals (Tappel, 1972). Lipid peroxidation of polyunsaturated fatty acids particularly arachidonic acid results in the formation and accumulation of lipid peroxidation products known as lipofuscin pigments (Tappel, 1972). These pigments accumulate in tissues (myocardium, brain, liver, testicles, etc.) in response to vitamin E deficiency, oxidative stress and age. Results of chemical analyses suggest that these lipofuscin pigments arise from membrane lipid and protein fragments formed in the course of their peroxidation phenomena (Porta and Hartroft, 1969).

Consequences of lipid peroxidation are alterations in structural (membrane permeability), functional components of the cell and their component enzymes particularly subcellular particles such as mitochondria, microsomes, and lysosomes.

Role of α-Tocopherol as a Chain Breaking Antioxidant

A major biological function of α-tocopherol is to act as a chain breaking antioxidant, scavenging free radicals, capable of terminating chain reactions among unsaturated fatty acids (Zalkin and Tappel, 1960).

Chain-breaking antioxidants react directly with radicals. Many phenols (ArOH) of which vitamin E is an example, can stop a radical chain reaction because they are able to trap the chain-carrying peroxyl radicals. The free radical scavenger action of α-tocopherol depends on the ability of donation of the phenolic hydrogen atom to a fatty acyl free radical, which resolves the unpaired electron of the radical so that free radical attack on further molecules of unsaturated fatty acids is prevented (Burton, 1989)

$$\mathrm{ArOH + ROO \rightarrow ROOH + ArO^{\cdot}}$$

The relatively stable phenoxyl radical produced is so unreactive to attack another polyunsaturated fatty acid moeity or O_2 that instead it is inactivated by reaction with a second peroxy radical $\mathrm{ROO^{\cdot}}$ + $\mathrm{ArO} \rightarrow$ inactive products or may in the presence of ascorbate undergo reduction back to the original phenol (Burton, 1989).

The latter mechanism is the basis of the so called synergistic action of vitamins C and E. The antioxidant breaks the chain of oxidation and can dramatically reduce the length of the autoxidation chain and the extent of peroxidative damage. The better the antioxidant, the greater is the amount of material that is spared from peroxidative damage. Vitamin E appears to be a factor ensuring the integrity of cellular membranes protecting their constituent polyunsaturated fatty acids from peroxidation.

CHAPTER 3
PLASMA AND TISSUE LEVELS OF VITAMIN E IN SHEEP FOLLOWING INTRAMUSCULAR ADMINISTRATION IN AN OIL CARRIER

Introduction

Vitamin E is a biological antioxidant that has been used pharmacologically in young sheep and cattle for the prevention or treatment of conditions associated with nutritional muscular dystrophy (Jenkins et al., 1972). Assessments of the vitamin E status in the ruminant animal treated with various tocopherol preparations have relied on serum or plasma tocopherol concentrations (Jenkins et al., 1970). To our knowledge there is no information on the effects of various forms of vitamin E preparations on sheep tissue tocopherol levels. The purpose of the present investigation was to assess the bioavailability in sheep of two forms of vitamin E (d-α- and dl-α-tocopherols) administered intramuscularly using a serial blood plasma analysis of α-tocopherol concentration. In addition, the α-tocopherol levels were determined in a few selected tissues.

Experimental

Animals

Yearling crossbred wethers weighing 40-50 kg were used. All animals originated from a flock that was born and raised in total confinement (Heaney et al., 1980). The animals were fed *ad libitum*, a diet that consisted of providing grass (40%), hay (40%), and corn silage (20%). The trial was carried out in three successive periods (2 months each), with each period utilizing one group of five sheep. Sheep were administered respective tocopherol treatments intramuscularly. The parenteral vitamin E preparations, suspended in sesame oil (200 mg/ml) were administered slowly at 40 mg/kg body weight into the muscle of the crural area of each leg. Treatments with respective slaughter times are as follows:

Group 1 - dl-α-tocopherol at 240 h
Group 2 - d-α-tocopherol at 360 h
Group 3 - d-α-tocopherol at 240 h

A group of six wethers originating from the same flock, was used as a control (Group 4).

Blood samples were taken from the jugular vein of treated sheep at specific time intervals after dosing ranging from zero to 360 h. The plasma was separated from the cellular blood components in a refrigerated centrifuge at 4°C and stored at -20°C until assayed for α-tocopherol. Tissue samples were taken from all sheep after slaughter and stored at -20°C until analyzed for α-tocopherol content. Feed samples were taken during the experiment for vitamin E analysis.

Analytical

High pressure liquid chromatography (HPLC) was used for plasma α-tocopherol determination (McMurray and Blanchflower, 1979a). The HPLC system consisted of an M 6000 pump and U6K septumless injector (Waters Associates Inc., Milford, Mass., USA) and Waters μ Bondapak C_{18} (10 μm) column (3.9 x 300 mm) coupled to a Perkin-Elmer 650-10S fluorescence spectrophotometer set at 295 and [illegible] (excitation) and emission, respectively. The column was eluted with methanol water (97:3) at 3 min/ml. All solvents were HPLC or pesticide grade. Blood plasma (2 ml) samples were precipitated with ethanol (2 ml), and extracted with hexane (4 ml). Tissue samples were saponified and extracted with hexane (Thompson and Hidiroglou, 1983).

Identification and quantitation of the α-tocopherol was accomplished by comparison of retention time and peak areas with d-α-tocopherol standard. Tocopherol determination in roughage was performed according to the techniques of McMurray and Blanchflower (1979b).

Statistical Analysis

The indexes of bioavailability of the various preparations of vitamin E were determined by analysis of variance. These indexes were: 1) the maximum α-tocopherol plasma (C max) concentration (peak of plasma α-tocopherol concentration curve), 2) the time of maximum (t max) α-tocopherol concentration, and 3) the area (AUC) under the α-tocopherol plasma concentration time curve (Balard, 1968, Koch-Weser, 1974). Differences in the vitamin E concentration between corresponding tissues in the four groups were determined by analysis of variance.

Results

Tocopherol Content of Roughages

Average tocopherol content of roughage at the time of consumption were 39, 68 and 34 (mg/kg DM) for maize-silage, grass-silage and hay, respectively. The only forms of vitamin E found in hay and grass-silage were α tocopherol. In maize-silage α-tocopherol was, on the average about half the total tocopherol and was equal to the sum of γ + β tocopherols (26%) and δ tocopherol (24%).

Blood Plasma α-Tocopherol

After IM administration of d-α-tocopherol preparation, a lag period was noted in the sheep until vitamin E increased in the systemic circulation (Fig. 3,1; Group 2). There was no clear evidence of an elimination phase in the data. In all 3 groups there was a suggestion of a plateau near the peak. It would appear as though the system reached an equilibrium at or near peak levels.

In Group 2 (IM d-α-tocopherol injected sheep killed 360 hr after dosing) the most important index of bioavailability (Koch-Weser, 1979), the area under the plasma concentration time curve (AUC) was the largest ($P<0.01$) compared to the other 2 groups (Table 3,1). This appeared to be due to a more sustained release into the plasma (t max much larger), resulting in a higher delivery of the vitamin. Maximum concentration (C max) was much higher ($P<0.01$) in group 2 than in the others.

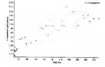

FIGURE 3.1. MEAN PLASMA α-TOCOPHEROL LEVELS IN SHEEP AFTER SINGLE I.M. INJECTION OF D-α-TOCOPHEROL SUSPENDED IN STERILE SESAME OIL

TABLE 1.1. D-α-TOCOPHEROL PHARMACOKINETIC PLASMA VALUES IN SHEEP AFTER A SINGLE INTRAMUSCULAR ADMINISTRATION OF VITAMIN E (40 mg/kg).

Group	Vitamin E preparations	Cmax[1] (μg/ml)	Tmax[2] (hr)	Co[3] (μg/ml)	Cmax/Co (μg/ml)	Ct[4] (μg/ml)	Ct/Co	Cmax/Ct (μg/ml)	AUC[5] (μg/ml/hr)
1	dl-α-tocopherol	1.76^d	151^b	1.12^c	1.68^d	1.47^b	1.39^d	1.23	286^d
2	d-α-tocopherol	2.40^c	254^c	0.94^d	2.68^c	1.87^a	2.06^c	1.35	637^c
3	d-α-tocopherol	1.84^d	190^a	0.71^d	2.63^c	1.30^b	1.82^c	1.54	277^d
	SE	0.23	24.78	0.09	0.21	0.17	0.24	0.15	27

Sheep in Group 1 and 3 were slaughtered 240 h after dosing while sheep in Group 2 were killed 360 h after dosing.

[1] Maximum plasma concentration.

[2] Maximum time (hr).

[3] Initial plasma concentration.

[4] Terminal plasma concentration

[5] Area under plasma concentration-time curve.

[a,b] Means in the same column with different superscripts differ (P<0.05).

[c,d] Means in the same column with different superscripts differ (P<0.01).

In Group 3 (IM d-α-tocopherol injected sheep killed 240 hr after dosing) there was also a more sustained delivery than in group 1 (t maximum 191>151) This resulted in a marginal increase in C max but no increase in AUC was noted. This may be due to the fact that initial values in Group 3 were very low compared to the other groups (C_0=0.71). It was noted that relative to initial values the increase in vitamin E concentration in Group 3 sheep was much higher than for Group 1 and approached the relative increase for Group 2 (C max/Co=2.63).

The AUC measurement of Group 3 was comparable to that of group 1 This may have been due largely to the higher initial value in this group. The elimination rates of the three groups appeared to be very similar (no differences in Cmax/C_T)

Tissue Concentrations of Vitamin E

Sheep receiving d-α-tocopherol and slaughtered 360 hr after dosing had higher (P<0.05) pancreatic, hepatic, lung, spleen and muscle α-tocopherol levels than the control group (Table 3.2). There also was higher α-tocopherol concentration (P<0.05) in the pancreas in sheep of Group 2 than Group 3. In the lung brachiocephalicus muscle, pancreas and spleen of sheep slaughtered 240 hr after dosing, higher α-tocopherol concentrations were found in the d-α-tocopherol than dl-α-tocopherol administered animals In these tissues α-tocopherol concentrations in Group 3 had a tendency to be higher than those of Group 1 sheep α-tocopherol concentrations in all groups were higher (P<0.01) in the adipose and adrenal than in other tissues analyzed.

TABLE [illegible]. α-TOCOPHEROL LEVELS IN THE TISSUES (µg/g wet)[1] OF SHEEP FOLLOWING A SINGLE INTRAMUSCULAR ADMINISTRATION (40 mg/kg BODY WEIGHT) OF VARIOUS VITAMIN E PREPARATIONS

Groups: Vitamin E preparation: Mode administration: Time after dosing (hr)	dl-α-tocopherol 240	d-α-tocopherol 360	d-α-tocopherol 240	Control
Tissues:				
Adrenal	11.52±4.29^{ab}	13.62±1.31^{ab}	12.49±1.99^{ab}	17.64±6.36^{ab}
Depot fat	10.52±1.73^{ab}	17.85±1.69^{ab}	16.82±2.52^{ab}	12.84±1.68^{ab}
Muscle vastus medial	4.20±0.55^{b}	7.75±0.60^{a}	4.92±1.01^{b}	3.84±0.94^{b}
Muscle brachiocephalicus	2.74±0.41^{d}	6.26±1.13^{c}	5.77±0.84^{c}	3.46±0.70^{d}
Heart	3.36±0.40^{ab}	5.33±1.03^{ab}	3.84±0.37^{ab}	5.76±1.59^{ab}
Kidney	2.87±0.58^{ab}	6.10±0.93^{ab}	4.57±0.99^{ab}	3.44±0.91^{ab}
Lung	3.06±0.71^{e}	7.52±1.10^{c}	5.46±0.76^{d}	2.91±0.23^{e}
Liver	4.27±0.37^{a}	5.92±1.39^{a}	4.18±0.48^{a}	2.22±0.36^{b}
Pancreas	3.04±0.56^{b}	6.94±1.00^{a}	6.34±1.22^{a}	4.32±0.50^{b}
Spleen	2.72±0.56^{b}	6.08±1.04^{a}	5.65±1.04^{a}	3.30±0.54^{b}

---

[1] Least square means ± SE.

[a,b] Means in the same rows with different superscripts differ (P<0.05).

[c,d,e] Means in the same rows with different superscripts differ (P<0.01).

Discussion

Blood plasma vitamin E data showed that in sheep dosed with various vitamin E preparations, the movement of the active free α-tocopherol from the site of the IM injection to the blood stream was quite slow. In all sheep given the vitamin E preparation by IM oil depot injection, a delayed increase in plasma α-tocopherol levels occurred. D-α-tocopherol IM administration proved to be more potent than dl-α-tocopherol. The length of the experimental period had an effect on the bioavailabilities index. The IM dosed sheep with d-α-tocopherol and slaughtered 360 hr later had a bioavailability much higher than in sheep dosed similarly but killed 240 hr after dosing. During this 360 hr period a gradual but sustained response occurred.

Overman et al (1954) reported that IM administration of vitamin E is ineffective in humans, regardless of the type of compound and the type of vehicle (oil or water) used. However, according to Caravaggi et al. (1968), IM injection of vitamin E is the best route for administration of this vitamin. These workers observed that in sheep, following IM injection of a commercial preparation of d-α-tocopherol acetate in aqueous suspension, the plasma α-tocopherol reached its peak 8 hr after its administration. The slow release of the IM injected vitamin E preparation in oil carrier could be due to myositis and inflammation of the lymphatic vessels (Dilie et al., 1976), through which the vitamin E molecule is absorbed (Blomstrand and Forsgren, 1968; Machlin et al., 1979). It is also known (Yoffey and Courtice, 1956) that exercise influences the flow of fluid along the lymphatic vessels. When an animal is at rest, as in these experiments, there is little or no lymphatic flow, and this would affect the release of the vitamin E preparations which probably was confined to connective tissue surrounding the muscle (Baird, 1968). Bille et al. (1976) showed that vitamin D injected by IM oil depot, into slaughter pigs, had a half-life of about 30-40 days at the injection site, and that oil granulomata had formed after dosing. Recently, it was reported (Dickson et al., 1986) that in sheep, following the IM injection of a vitamin E preparation (5 ml) suspended in arachis oil (400 mg dl-α-tocopherol acetate/ml) into quadriceps muscle, a considerable storage of vitamin E occurred in their lymph gland (iliac). These workers observed that in the injected sheep a continuous increase of vitamin E concentration occurred during 3 weeks (from 76 µg/g to 2687/µg/g lymph gland). On weeks 5 and 6 there were still high concentrations of vitamin E (1855 µg and 550 µg/g) in the lymph gland (iliac) of the killed sheep. The results of their experiment showed that the duration of the experimental period was relatively short and longer time was needed in order to establish accurately the various indexes of bioavailability. The planning of our experiment was based on work carried out on chicks (Marusich et al., 1967) and in dogs (Newmark et al., 1975) dosed IM with various forms of vitamin E. In their animals the plasma vitamin E showed its peak within 24 hr. The difference in initial d-α-tocopherol plasma values between the present 3 groups may have been due to different roughage batches used before the start of the trial. The results reported herein show that the time of slaughter had a major influence on tissue vitamin E levels. In sheep slaughtered 360 hr after IM administration of d-α-tocopherol, tissue levels of α-tocopherol were higher than these of control. In some cases there were large individual variations in tissue α-tocopherol concentrations which made comparisons difficult between corresponding tissues in the various groups. It is apparent that of the tissues examined the depot fat is the preferred storage tissue for vitamin E. According to Machlin et al. (1979) adipose tissue tocopherol is not readily available upon metabolic demand. Tocopherol concentrations in the liver of control were low indicating low dietary intake. Rammell and Cunliffe (1983) using HPLC reported that levels of 2 mg of α-tocopherol per kg of fresh liver were observed in clinically normal sheep. According to these workers α-tocopherol hepatic levels less than 2 mg/kg became health problems. In deer, Meadows et al. (1982), also using HPLC, observed liver α-tocopherol concentrations of 5 μg/g wet tissue.

In conclusion, the results showed a delayed increase in plasma α-tocopherol concentration in sheep administered IM two vitamin E preparations. A more sustained increment in plasma α-tocopherol was observed in sheep IM-injected with d-α-tocopherol than dl-α-tocopherol. Following d-α-tocopherol IM administration, higher tissue α-tocopherol levels occurred in sheep killed 360 hr after dosing than in those of control sheep.

Summary

The bioavailability of two vitamin E preparations (d-α-tocopherol and dl-α-tocopherol) suspended in sesame oil solution and administered intramuscularly was evaluated in sheep. In sheep administered d-α-tocopherol and killed after 360 hr (Group 2), there was a higher ($P<0.01$) bioavailability as assessed by the area under the plasma concentration-time curve, than for sheep killed 240 hr after injection with d-α-tocopherol (Group 3) or dl-α-tocopherol (Group 1). In the 3 groups (5 sheep each) the highest blood plasma α-tocopherol increment occurred in the d-α-tocopherol injected sheep. For sheep injected with d-α-tocopherol and slaughtered at 360 hr (Group 2) the pancreatic, hepatic, lung, spleen and muscle tocopherol concentrations were higher ($P<0.05$) than in the control group. Also in group 2 there was a tendency for higher tissue tocopherol concentrations than in the other vitamin E treated sheep (Group 1 and 3).

CHAPTER 4
BIOAVAILABILITY OF VARIOUS VITAMIN E COMPOUNDS IN SHEEP

Introduction

The biological activity of the various tocopherol compounds has been established from experiments with laboratory animals. However, biopotency evaluation of different forms of vitamin E based on a specific animal model (i.e., rat antisterility test) may not be valid for other species. Biopotencies based on this test do not correlate with human data. Recently Baker et al. (1986) reported that human assay data confirmed the currently accepted biopotencies of 1.0 IU/mg and 1.36 IU/mg for dl-tocopheryl acetate and d-α-tocopheryl acetate, respectively. There are little data available for ruminants on the absorption and body distribution of vitamin E. In view of the unique digestive and physiological system in ruminants, the present investigation determined bioavailability of the various forms of vitamin E in sheep fed over a relatively long-term study (28 days).

Materials and Methods

Initially, twenty crossbred yearling wethers weighing 60 to 70 kg were used. All animals originated from a flock fed a commercial mix that consisted of 56.45% corn meal, 16.5% (44% CP) soybean meal, 25% cottonseed hulls, 1% trace mineral salt, dicalcium, 1% monocalcium phosphate, and .037% of vitamin A and D_3. They were fed this diet throughout the experiment.

Sheep were placed in individual metabolism cages 10 days before administration of vitamin E as an adjustment period. They were randomly allotted to four dietary groups of five each that consisted of 400 International Units/day/sheep of either (1) dl-α-tocopherol, (2) dl-α-tocopheryl acetate, (3) d-α-tocopherol (4) d-α-tocopheryl acetate, which was mixed with the commercial diet. Appropriate amounts of the various forms of tocopherols were dissolved in absolute ethanol prior to diet mixing. In the middle of the experiment, one sheep from group 3 and two sheep from group 4 stopped eating and were removed from the experiment.

Blood samples were taken at various intervals by jugular puncture with plasma separated from the cellular blood components in a refrigerated centrifuge and stored at -30°C until assayed for vitamin E. All animals were slaughtered after 30 days. Samples from selected tissues (fat, muscles from the neck and leg, kidney, lung, heart, spleen, liver, pancreas) were collected and stored at -30°C until analyzed for d-α-tocopherol.

Analytical Methods

Quantitation of d-α-tocopherol in sheep tissues, as well as plasma samples, was performed by high-pressure liquid chromatography (HPLC) using a fluorescent detector, following tissue homogenization and heptane extraction. Identification and quantitation of α-tocopherol were obtained by comparison of retention time as well as peak areas with tocopherol standards. Standards were purchased from Eastman Kodak (Rochester, NY, USA).

Instrumentation

The chromatographic apparatus consisted of an M 6000 pump and a WK septumless injector (Waters Associates Inc., Milford, Mass., USA). A Perkin-Elmer 650-150 fluorescence spectrophotometer, equipped with a microflow cell unit, was used for quantitation. Wavelength settings were 295 and 330 nm for excitation and emission, respectively. The column was a µBondapak C18 (3.9 mm x 30 cm) of 10 µm particle size purchased from Waters Associates (Milford, Mass., USA). The mobile phase was a solvent system consisting of methanol and water in a 97:3, with a flow rate of 3 ml/minute. All solvents used were HPLC grade.

Mass Spectrometry in Combination with Gas Chromatography

Further identification of α-tocopherol in various tissues was carried out by a combination of a Hewlett-Packard (HP) gas chromatograph 5790-mass spectrometer 5970A series with a data station (HP-9000). The mass spectrometer performs three basic functions. First, molecules are subjected to bombardment by a stream of high-energy electrons, converting some of the molecules to ions. The ions are accelerated in an electric field. Second, the accelerated ions are separated according to their mass-to-charge ratio in a magnetic field or electric field. Finally the ions with a particular mass-to-charge ratio are detected by a device which is able to count the number of ions which strike it. The detector's output is amplified and fed to a recorder (Roboz, 1968). Eluates from the HPLC were purified for α-tocopherol by thin layer chromatography on silica gel using cyclohexane-diethylether (4:1 v/v) as the solvent.

Authentic standards of d-α-tocopherol, as well as tissue samples, were silylated before injection onto the GC/MS (Ingold et al., 1987)

The tocopherol trimethylsilyl ethers were injected (3 μl) into a 12 m x 2 mm i.d. ultra-1 (OV 101 methyl silicone) capillary column maintained at 280°C with an injector temperature of 350°C and a helium carrier gas flow rate of 0.5 cc/min, which was connected to a series mass selective detector. Mass spectra were obtained at an electron beam energy of 70 eV and an accelerating voltage of 1800 volts. Molecular ions ranging from 50 to 600 mass/charge were monitored continuously.

Statistical Methods

Indices of bioavailability of various preparations of vitamin E were performed by analysis of variance. These indices were: 1) the maximum α-tocopherol plasma concentration (peak of plasma α-tocopherol concentration curve), 2) the time of maximum (t max) α-tocopherol concentration, and 3) the area (AUC) under the α-tocopherol plasma concentration time curve. Analysis of variance was also used to estimate the difference in the vitamin E concentrations between corresponding tissue in the four groups, as well as among tissues.

Results

Tissues

Diet and tissues were highly significant ($P < 0.0001$) in their vitamin E concentrations, but diet x tissue interaction was not significant ($P > 0.05$) (Table 4.1). Higher α tocopherol concentrations ($P < 0.01$) were observed in the tissues of sheep fed the d-α-tocopherol than other vitamin E compounds (Table 4.2). Analysis of variance for each individual tissue of the four diets showed differences ($P < 0.01$) only for heart, kidney and lung (Figure 4.1). Higher concentrations (μg/g fresh tissue) of α-tocopherol were observed in the sheep's heart fed d-α-tocopherol (14.74 ± 1.64 SE) than in the d-α-tocopheryl acetate (9.65 ± 1.64 SE), dl-α-tocopheryl acetate (8.47 ± 2.84 SE) or dl-α-tocopherol (6.20 ± 2.50 SE) supplemented sheep. Also, in d-α tocopherol fed sheep, higher concentrations were observed in lung (12.13 ± 2.58 SE) than in d-α-tocopheryl acetate (8.99 ± 1.51 SE) or dl-α-tocopheryl acetate (6.14 ± 1.16 SE) or dl-α-tocopherol (6.40 ±1.92 SE) supplemented sheep. In kidney, higher α tocopherol concentrations were found in the d-α-tocopheryl acetate (7.20 ± 0.27) than d-α-tocopherol (5.81 ± 0.67 SE) or dl-α-tocopheryl acetate (4.78 ± 1.84) or dl-α-tocopherol (4.11 ± 0.63). However, analysis of variance did not show a difference ($P > 0.05$) among corresponding tissues among treatments. In all diets, pancreas contained the highest concentrations, followed by liver and spleen. Immediately after these tissues, heart, lung and kidney were

TABLE 4.1. ANALYSIS OF VARIANCE FOR α-TOCOPHEROL CONCENTRATIONS IN TISSUES OF SHEEP FED DIFFERENT FORMS OF VITAMIN E.

Source	DF	MS	F	P
Model	35	142.76	16.10	0.0001
Error	117	8.86		
Corrected total	152			

Source	DF	SS	F	P
Diet	3	212.02	7.97	0.0001
Tissue	8	4318.39	60.86	0.0001
Diet x tissue	24	305.90	1.44	0.1049

TABLE 4.2. TISSUE α-TOCOPHEROL CONCENTRATIONS (μg/g FRESH TISSUE) IN THE FOUR DIETS.

Diet	Mean	N
d-α-tocopherol	10.43^a	36
d-α-tocopheryl acetate	8.75^b	27
dl-α-tocopheryl acetate	7.72^b	45
dl-α-tocopherol	7.46^b	45

In vertical row, numbers with the same letter are not different (P>0.05).

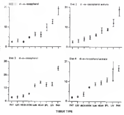

FIGURE 4.1 α-TOCOPHEROL CONCENTRATIONS IN THE VARIOUS TISSUES OF SHEEP SUPPLEMENTED WITH DIFFERENT VITAMIN E COMPOUNDS

ranked lower ($P<0.05$). Lowest ($P<0.05$) concentrations of α tocopherol were observed in the muscle and adipose tissue (Figure 4.1)

Table 4.3 (and Figure 4.2) shows that the relative retention time (minutes) of the trimethylsilyl esters (TMSi) obtained from purified α-tocopherol tissues samples are very similar to that obtained from the TMSi α-tocopherol standard.

Mass spectra of the gas chromatographic peak corresponding to the α tocopherol standard (TMSi) (Figure 4.3) and that corresponding to the peak with the same retention time obtained for the pancreas, for example, are identical, confirming the presence of α-tocopherol in these biological extracts

The peak obtained at mass/charge 502 represents the molecular ion of α-tocopheral TMSi (430 α-tocopherol + 72 trimethylsilylether). The fragmentation pattern reveals peaks at mass/charge 277, resulting from the loss of the side chain ($C_{16}H_{33}$) and at mass/charge 237 due to the cleavage of the chroman structure accompanied by hydrogen rearrangement and the loss of a CH_3-C≡CH fragment (Nair and Luna, 1968)

Blood Plasma

Analysis of blood plasma α-tocopherol concentrations summarized in Table 4.4 show that diet and tissue source were significant ($P<0.01$) but not the interaction diet x day. Higher ($P<0.01$) α-tocopherol plasma concentrations were observed in sheep supplemented with d-α-tocopherol on days 9, 15, 17 and 25 (Figure 4.4) than in the other three groups. Also, in sheep fed d-α-tocopherol, the most

TABLE - 3 RETENTION TIME OF α TOCOPHEROL FROM GAS-CHROMATOGRAPHY/MASS SPECTROMETRY.

Relative retention time (minutes) Substance	TMSi[a]
Standard α-tocopherol	4.1
Pancreas	3.9
Liver	3.8
Heart	3.9
Spleen	3.9
Lung	3.9

[a] TMSi, Trimethylsilyl ether.

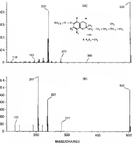

FIGURE 4.2. IDENTIFICATION OF α-TOCOPHEROL BY GAS-CHROMATOGRAPHY/MASS SPECTROPHOTOMETRY (A) α-TOCOPHEROL STANDARD, (B) α-TOCOPHEROL IN PANCREAS

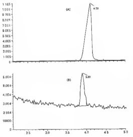

FIGURE 4.3. MASS SPECTRAL SCAN OF THE α-TOCOPHEROL STANDARD (A) AND α-TOCOPHEROL IN THE PANCREATIC TISSUE (B) FOLLOWING HPLC COLLECTION

TABLE 4.4. ANALYSIS OF VARIANCE OF α-TOCOPHEROL CONCENTRATIONS IN BLOOD PLASMA OF SHEEP FED DIFFERENT FORMS OF VITAMIN E.

Source	DF	MS	F	P
Model	66	2.99	4.64	0.0001
Error	217	0.64		
Corrected total	283			

Source	DF	SS	F	P
Diet	3	44.79	23.09	0.0001
Tissue	16	225.13	11.13	0.0001
Diet x tissue	47	40.53	1.33	0.0883

TABLE 4.5. AREA (AUC) UNDER THE PLASMA α-TOCOPHEROL CONCENTRATION CURVE (μg/ml h^{-1}).

Diet	AUC (mean ± SE)
d-α-tocopherol	102.4 ± 8.13^{a}
d-α-tocopheryl acetate	90.0 ± 9.44^{b}
dl-α-tocopheryl acetate	75.0 ± 7.31^{c}
dl-α-tocopherol	67.3 ± 7.31^{d}

In vertical row, numbers with the same letter are not different (P>0.05)

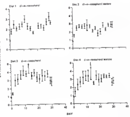

FIGURE 4.4. α-TOCOPHEROL CONCENTRATIONS IN THE BLOOD PLASMA OF SHEEP SUPPLEMENTED WITH DIFFERENT VITAMIN E COMPOUNDS

important indice of bioavailability, the area under the plasma α-tocopherol concentration time curve (AUC), was larger ($P<0.05$) as compared to dl-α-tocopheryl acetate or dl-α-tocopherol supplemented sheep (Table 4.5). No statistical difference ($P>0.05$) was observed in the maximum α-tocopherol concentration (µg/ml) between the four groups. They were 3.6 ± 0.69 (SE) (dl-α-tocopherol) and 4.3 ± 0.69 (dl-α-tocopheryl acetate), 5.11 ± 0.77 (d-α-tocopherol) and 4.8 ± 0.89 (d-α-tocopheryl acetate). Also, no difference on the time (days) of maximum concentration ($P > 0.05$) was observed. They were 18.2 ± 3.24 (SE) (dl-α-tocopherol), 14.8 ± 3.24 (dl-α-tocopheryl acetate), 11.5 ± 4.30 (d-α-tocopherol) and 22.3 ± 4.96 d-α-tocopheryl acetate. The elimination curve rates (concentration maximum/concentration terminal) in the four groups were very similar. They were 1.6 ± 0.20 (SE) (dl-α-tocopheryl acetate), 1.8 ± 0.22 (d-α-tocopherol) and 1.7 ± 0.25 (d-α-tocopheryl acetate) and 1.4 ± 0.20 (dl-α-tocopherol).

Discussion

Paucity of bioavailability data from various forms of tocopherol in sheep limits any comparison with the present data. Biological response of sheep to dietary intake of various forms of vitamin E was greater with the d-α-tocopherol than with its esterified form or the isomers. Indeed, the area plasma time curves, which provide a proper measure of relative availability for physiological function, and tissue α-tocopherol concentrations were highest in the d-α-tocopherol supplemented group. This indicates that in the ruminant body there may be a difference in the transfer rate between natural and unnatural forms of vitamin E (Dunkley et al., 1967). As suggested in humans (Week et al., 1952), it could be that the physiological mechanism effecting hydrolysis in the ruminant duodenum of tocopherol esters is less effective than in rats. It is reasonable to assume that plasma tocopherol levels in this experiment reflect the relative efficacy of various forms of vitamin E on tissue storage. Indeed, as was reported for humans (Dju et al., 1958), the present data on α-tocopherol concentrations in sheep tissue show a variable pattern which is largely a reflection of varied bioavailability of the different forms of vitamin E. It may be concluded that following ingestion of equivalent amounts (IU) of the various forms of tocopherol by sheep d-α-tocopherol was more potent than d-α-tocopheryl acetate or the l-isomeric forms of vitamin E.

Summary

Seventeen crossbred yearling wethers were randomly allotted to four dietary groups that received 400 IU/day/sheep either of (1) dl-α-tocopherol (five sheep), (2) dl-α-tocopheryl acetate (five sheep), (3) d-α-tocopherol (four sheep) or (4) d-α-tocopheryl acetate (three sheep). Blood samples were taken at day 0 and then at frequent intervals for α-tocopherol determination. At the end of the 28-day experiment, animals were killed and various tissues sampled. Higher concentrations of α-tocopherol were observed in tissues of sheep fed d-α-tocopherol than the other tocopherol forms. In sheep fed d-α-tocopherol, the most important index of bioavailability, the area under the plasma α-tocopherol time curve (AUC) (Gibaldi and Perrier,

1975) was larger as compared to other forms of vitamin E supplementation.

CHAPTER 5
BLOOD PLASMA AND TISSUE CONCENTRATIONS OF VITAMIN E IN BEEF CATTLE AS INFLUENCED BY SUPPLEMENTATION OF VARIOUS TOCOPHEROL COMPOUNDS

Introduction

Biopotencies of various tocopherol compounds are generally provided by the following relationship according to the National Formulary: 1 mg dl-α-tocopheryl acetate = 1.0 IU, 1 mg dl-α-tocopherol = 1.1 IU; 1 mg d-α-tocopheryl acetate = 1.36 IU; 1 mg d-α-tocopherol = 1.49 IU. These values have been based largely on small animal bioassay (rat anti-sterility assay). Horwitt (1980) and Horwitt et al. (1984) reported that in humans, on a mg basis, d-α-tocopheryl acetate was 2.16 times more potent than dl-α-tocopheryl acetate following a single oral dose. However, Baker et al. (1986) confirmed in humans following a continuous oral dosing that 1 mg of dl-α-tocopheryl acetate (all-racemic-α-tocopheryl acetate) has a biopotency of 1.0 IU whereas d-α-tocopheryl acetate (2R, 4'R, 8'R-α-tocopheryl acetate) has a biopotency of 1.36 IU (28 d). Horwitt (1980), Horwitt et al. (1984) and Baker et al. (1986) used the elevation of plasma α-tocopherol as a measure of bioavailability.

Although much is known about clinical signs of deficiency in calves and lambs (Rice and McMurray, 1982) very little attention is given to the different potencies of various vitamin E forms in cattle. The present authors (Hidiroglou and McDowell, 1987; Hidiroglou et al., 1988) have undertaken a series of studies on the utilization of different chemical forms of vitamin E by sheep. That work was extended to cattle with the purpose of comparing plasma and tissue α-tocopherol concentrations following supplementation of various tocopherol compounds.

Materials and Method

Animals

Twenty-four Charolais-Hereford crossbred beef cows ranging from 2 to 10 years of age were culled 6 months after calving from the herd of the Nappan Experimental Farm, N.S., Canada. These unbred cows were stratified by age into four groups, and the six animals within each group were randomly assigned to four dietary tocopherol preparations. The treatments were d-α-tocopheryl acetate, d-α-tocopherol, dl-α-tocopherol and dl-α-tocopheryl acetate. Cows received daily 1,000 IU (a dosage above a physiological level) of their respective vitamin E preparation. This amounted to 735 mg of d-α-tocopheryl acetate, 671 mg of d-α-tocopherol, 900 mg of dl-α-tocopherol and 1000 mg of dl-α-tocopheryl acetate, respectively. Each vitamin E preparation was diluted in alcohol to 3 ml and mixed with 25 g of dry molasses. All cows were individually fed 2 kg of average quality grass hay (8.7% CP, 35% ADF, 54% TDN, .51% Ca, .23% P, .02 ppm Se and 12 ppm vitamin E) and had ad libitum access to a barley-soybean mixture (Table 5.1) with no added α-tocopherol. The vitamin E-molasses mixture was prepared daily and was topdressed on the grain portion for each individual cow. The experiment lasted 28 days after which time the cows were sacrificed at commercial facilities.

TABLE 5.1 COMPOSITION OF THE GRAIN PORTION OF THE DIET FED TO COWS FOR 28 d[a]

Ingredient	% as-fed basis
Barley	90
Soybean meal	7.5
Calcium carbonate	1.0
Dicalcium phosphate	1.0
Vitamin premix[b]	.25
Trace mineral salt[c]	.25

[a] Analyzed for vitamin E and selenium (8 ppm and .235 ppm, respectively).

[b] Provided, per kilogram of diet: 5,000 IU vitamin A and 500 IU vitamin D.

[c] Provided, per kilogram of diet: 2.1 g NaCl, .22 mg I, .088 mg Co, 9 mg Fe, .725 mg Cu, 2,625 mg Mn and 8.75 mg Zn.

Blood Collection

Blood samples (15 ml) were obtained via jugular venipuncture on d 0, 1, 7, 14 and 28 of the feeding period and plasma was analyzed for α-tocopherol concentration. Samples were always collected in the morning before vitamin E treatments were provided. Blood samples were centrifuged at 1,000 x g for 15 min at 4°C immediately after sampling, and plasma was stored at -20°C until analyzed for α-tocopherol concentration.

Tissue Collection

On the last d of the experiment, cows were shipped to a local abattoir and slaughtered the following d. At that time, 10-50 g samples were collected from ten different tissues: heart, thyroid, liver, kidney, adrenal, pancreas, spleen, lung and neck muscle. These samples were frozen within 2 hours and stored at -20°C until analyzed for α-tocopherol concentration using high pressure liquid chromatography.

Analytical Method

Quantification of α-tocopherol in tissues was performed by HPLC using a fluorescence detector as described by McMurray and Blanchflower (1979b) following tissue homogenization and heptane extraction by the method of Burton et al. (1985) and Ingold et al. (1987). Serum samples were prepared for HPLC analysis according to the method of McMurray and Blanchflower (1979b). Identification and quantification of α-tocopherol were by comparison of retention times and peak areas with tocopherol standards. Selenium and α-tocopherol in the feed were determined, respectively, by the method of Hoffman et al (1968) and McMurray and Blanchflower (1979a).

Instrumentation

The chromatographic apparatus consisted of a M 6,000 pump and WK septumless injector. A Perkin-Elmer 650-150 fluorescence spectrophotometer equipped with a microflow cell unit was used for quantification. Wavelength settings were 295 and 330 nm for excitation and emission, respectively. The column was a µ Bondapak C18 (3.9 mm x 30 cm) of 10 µm particle size. The mobile phase was a solvent system (HPLC grade) consisting of methanol and water (97:3) with a flow rate of 3 ml/min.

Mass Spectrometry in Combination with Gas Chromatography

Further confirmation of α-tocopherol in various tissues was carried out by a combination Hewlett-Packard (HP) gas chromatograph 5790-mass spectrometer 5970A series with a data station (HP-9000). Eluate from the HPLC was purified for α-tocopherol by TLC on silica gel using cyclohexane-diethylether (4:1 v/v) as the solvent. Authentic standards of d-α-tocopherol as well as tissue samples were silylated after HPLC as described by Ingold et al. (1987) before injection onto the gas chromatograph/mass spectrometer. The tocopherol trimethylsilyl ethers were injected (3 µl) into an ultra-1 (OV 101 methyl silicone) capillary column (12-m x 2-mm i.d.), maintained at 280°C with an injector temperature of 350°C and a helium carrier gas flow rate of .5 cc/min, which was connected to a series mass selective detector. Mass spectra were obtained at an electron beam energy of 70 eV and an accelerating voltage of 1,800 volts. Molecular ions ranging from 50 to 600 mass/charge were monitored continuously.

Statistical Method

Analysis of variance (Steel and Torrie, 1980) was used to estimate the difference in α-tocopherol concentration among similar time blood samplings as well as among corresponding tissues in the four treatment groups. An additional two way analysis was carried out to test groups and d for blood plasma and for groups and tissue types Comparisons were made by using Duncan's multiple range test (Duncan, 1985). Statistical models used in the experiment were as follows. For the one-way analysis: $Y_{ij} = \mu + g_i + e_{ij}$ where g_i is effect of i^{th} group; $Y_{ij} = \mu + d_i + e_{ij}$, where d_i is effect of i^{th} d. For two-way analysis: $Y_{ijk} = \mu + g_i + t_j + e_{ijk}$, where g_i is effect of i^{th} group and t_j is effect of j^{th} d.

Results

Plasma

Supplementation over a 1 to 7 d period of time with the various vitamin E preparations increased ($P < .01$) plasma α-tocopherol concentration above the baseline level (Table 5.2). Plasma α-tocopherol concentrations at 1 d were higher ($P < .01$) than the original baseline values in the d-α-tocopherol group but not for

TABLE 5.2. PLASMA CONCENTRATION OF α-TOCOPHEROL (μg/ml) IN CATTLE FED VARIOUS PREPARATIONS OF VITAMIN E

Item	Day 0	1	7	14	28	SE	Group mean
Treatment							
d-α-tocopheryl acetate	1.49^{ag}	2.46^{bfg}	3.67^{bef}	5.64^{bde}	7.65^{abd}	.71	4.18^{a}
d-α-tocopherol	1.39^{af}	3.82^{ae}	5.25^{ae}	8.41^{ad}	9.22^{ad}	.58	5.61^{b}
dl-α-tocopherol	1.39^{ag}	2.40^{bfg}	3.14^{bef}	3.98^{be}	5.44^{bcd}	.44	3.27^{c}
dl-α-tocopheryl acetate	1.37^{af}	2.65^{bef}	3.10^{bde}	4.02^{bde}	4.62^{cd}	.52	3.16^{c}
SE	.10	.25	.45	.82	.82		.27
Day mean	1.41^{d}	2.83^{e}	3.80^{f}	5.51^{g}	6.73^{h}	.31	

^{a,b,c}Means in the same column with different letters in their superscripts differ (P<0.01).

^{d,e,f,g}Means in the same row with different letters in their superscripts differ (P<0.01).

the other groups (P<.05). To elevate plasma α tocopherol concentration, d-α-tocopherol outranked all the other forms at all sampling dates except at 28 d, where its effect was not different (P>.05) from d-α-tocopheryl acetate (Table 5.2). D-α-tocopherol supplementation during the 28 d experimental period resulted in the highest blood plasma α-tocopherol concentration followed by d-α tocopheryl acetate, the racemic mixtures ranked lower (Table 5.2) despite being dosed at equal units. Blood plasma α-tocopherol increased continuously from 0 to 28 d (Table 5.2). The difference in plasma α-tocopherol concentration (μg/ml) between d 28 and d 0 were as follows (Table 5.2). D-α-tocopheryl acetate = 6.16; d-α-tocopherol = 7.83; dl-α-tocopherol = 4.05, dl-α-tocopheryl acetate = 3.25. Consequently the relative bioavailabilities of the various tocopherols vs dl-α-tocopheryl acetate (1.0 IU/mg) could be calculated as follows:

dl-α-tocopherol $\frac{(4.05 \times 1.1\ \text{IU/mg})}{3.25} = 1.37$ IU/mg; d-α-tocopheryl acetate $\frac{(6.16 \times 1.36\ \text{IU/mg})}{3.25} = 2.58$ IU/mg, d-α-tocopherol $\frac{(7.83 \times 1.1 \times 1.36\ \text{IU/mg})}{3.25}$ = 3.60 IU/mg.

Tissues

There were differences among treatments (P<.01) and types of tissue (P<.0001) in vitamin E tissue concentrations at slaughter 28 d after supplementation began Fig. 5.1 and Table 5.3). The adrenal gland and liver concentrations of α-tocopherol were greater (P<.01) for cattle fed d-α-tocopherol or d-α-tocopheryl acetate than when fed racemic forms of vitamin E. The α-tocopherol concentrations were

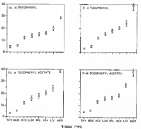

FIGURE 5.1. α-TOCOPHEROL CONCENTRATIONS IN THE TISSUES OF CATTLE SUPPLEMENTED WITH DIFFERENT VITAMIN E COMPOUNDS

TABLE 5 [illegible] TISSUE α TOCOPHEROL CONCENTRATIONS (μg/g FRESH TISSUE) [illegible] VARIOUS VITAMIN E PREPARATIONS

Tissue	Dietary form d-α-tocopheryl acetate	 d-α-tocopherol	 dl-α-tocopherol	 dl-α-tocopheryl acetate	SE	Tissue mean
	μg/g fresh tissue					
Adrenal gland	38.41^{a}	38.07^{a}	27.91^{b}	28.75^{b}	2.68	33.28^{d}
Heart	20.43^{a}	18.71^{a}	18.43^{a}	16.12^{a}	1.42	18.42^{f}
Kidney	12.07^{a}	13.11^{a}	8.81^{b}	12.28^{a}	1.00	11.56^{h}
Liver	24.20^{ab}	27.00^{a}	15.67^{c}	19.88^{bc}	1.79	21.68^{e}
Lung	15.73^{a}	16.52^{a}	10.64^{b}	15.04^{a}	1.08	14.48^{g}
Muscle	5.29^{a}	5.79^{a}	5.70^{a}	5.79^{a}	.46	5.64^{j}
Spleen	17.95^{a}	15.79^{ab}	11.76^{b}	13.88^{ab}	1.35	14.84^{g}
Thyroid	3.40^{b}	4.43^{ab}	5.63^{a}	4.35^{ab}	.46	4.45^{j}
SE	1.82	1.63	1.10	1.10		.80
Group mean	17.18^{a}	17.42^{a}	13.03^{b}	14.53^{b}	.57	

[a,b,c] Means in the same row with different letters in their superscripts differ ($P<0.05$).

[d,e,f,g,h] Means in the same column with different letters in their superscripts differ ($P<0.0001$).

lower in the kidney (P [illegible]) and lung ($P<.01$) of the cattle supplemented with dl-α-tocopherol than for cattle fed the other treatments. Higher concentrations ($P<.05$) of α-tocopherol were observed in the thyroid of cattle fed dl-α-tocopherol than in that of the cows receiving d-α-tocopheryl acetate, but the α-tocopherol concentrations were higher ($P<.05$) in the spleen of the group fed d-α-tocopheryl acetate than in that of the dl-α-tocopherol group. No difference ($P>.05$) was found for heart and muscle as a result of feeding the various forms of vitamin E.

Across all the treatments, the adrenal gland contained the highest α-tocopherol concentration, followed generally by the liver or heart, the lowest concentrations were observed in the thyroid and muscle tissues ($P<.05$) (Table 5.3 and Figure 5.1).

Identity of α-tocopherol in tissues was confirmed by comparing the mass spectra of the α-tocopherol standard to those obtained by collecting the eluates from the HPLC and introducing it into the gas chromatography/mass spectrometry system following silylation. A good spectral match was obtained for the sampled compared with the α-tocopherol standard spectra. Mass spectra of the gas chromatographic peak corresponding to the α-tocopherol standard (TMSi; Fig. 5.2) and that corresponding to the peak with the same retention time obtained for the plasma, for example, are identical, confirming the presence of α-tocopherol in these biological extracts. The peak obtained at mass/charge 502 represents the molecular ion of α-tocopherol (TSMi: 430 for α-tocopherol + 72 for trimethylsilyl ether). The fragmentation pattern reveals peaks at mass/charge 277 resulting from

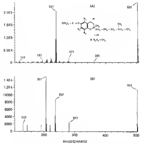

FIGURE 5.2 MASS SPECTRAL SCANNING OF THE α-TOCOPHEROL STANDARD (A) AND IN BLOOD PLASMA (B) FOLLOWING HPLC COLLECTION AND TLC.

the loss of the [illegible] of the chroman structure accompanied by hydrogen rearrangement and the loss of a CH_3-C≡CH fragment.

Discussion

The data show that, in cattle, a greater elevation of plasma levels of α-tocopherol occurred over pre-treatment levels after ingestion of the naturally occurring d-α-tocopherol than its ester forms or the l-epimer of α-tocopherol. These results agree with those of Baker et al. (1980) who reported in humans that plasma α-tocopherol was considerably higher when the free tocopherol was orally administered rather than its acetate ester. According to Machlin and Gabriel (1982), the higher blood plasma α-tocopherol attained following supplementation with α-tocopherol compared to α-tocopheryl acetate suggests that hydrolysis of the acetate may be a limiting factor when high levels of the vitamin are administered. Harris and Ludwig (1949) reported lower blood plasma α-tocopherol levels in humans dosed with the nonesterified rather than esterified forms. This is related to a diet-mediated oxidative destruction of the free form in the gut prior to its absorption. This does not seem to be the case with ruminants. Indeed, the stability of d-α-tocopherol in ruminal liquor is high (Astrup et al., 1974b). Vitamin E is not metabolized or oxidized to any great extent in the gastrointestinal tract of cattle (Astrup et al., 1974a).

Plasma pre-treatment α-tocopherol concentrations were low. Levels less than 1.5 μg/ml in blood plasma were considered by several workers as potentially hazardous for cattle (McMurray and Rice, 1982, Peterson and Hidiroglou, 1986). Following vitamin E administration the levels increased progressively for all treatments during the 4 wk experimental period to the range (4 to 9 μg/ml) reported acceptable for beef cattle (Bayfield and Mylrea, 1969).

In humans and monkeys, Machlin and Gabriel (1982) reported that it is advisable to treat subjects with high levels of α-tocopherol for at least 1 to 3 wk to attain maximal plasma α-tocopherol levels.

In order to evaluate the effectiveness of the various vitamin E preparations, most studies (Horwitt et al., 1984; Baker et al., 1986) have relied on serum α-tocopherol concentrations and have paid little attention to tocopherol concentrations in tissues. In the present experiment, tissue analysis showed higher concentrations following d-α-tocopherol or d-α-tocopheryl acetate supplementation than with the racemic forms. These results suggest strongly that d-α-tocopherol or its acetate form is more available to bovine tissue than the l-epimer. Cattle, like the rat, appear to discriminate well between 2R, 4'R, 8'R, and 2S, 4'R, 8'R α-tocopherol (Ingold et al., 1987).

Dju et al. (1958) reported that, in the human body, there is a wide variation in the distribution of α-tocopherol in the various tissues. In the present experiment, highest concentrations were detected in the adrenal gland and liver. The liver is the major storage organ for α-tocopherol (Machlin and Gabriel, 1982) and (for a short time at least) helps maintain plasma tocopherol levels when the intake of vitamin E becomes inadequate. As indicated earlier, in rats (Behrens and Madere, 1986), the adrenal gland accumulated more tocopherol per gram of tissue than other tissues, regardless of the source of vitamin E supplementation. In general the apparent biopotency of various vitamin E sources for cattle are comparable to results reported for humans by Horwitt et al. (1984).

Summary

Twenty-four crossbred beef cows were used to investigate the concentration of (α-tocopherol) in plasma and tissues following oral administration of four tocopherol sources. Animals were assigned to the following treatments: dl-α-tocopherol, d-α-tocopherol, dl-α-tocopheryl acetate and d-α-tocopheryl acetate. Animals received a daily oral dose of 1,000 IU of the respective tocopherol treatment for 28 d and then were slaughtered. Blood samples were collected on d 0, 1, 7, 14 and 28 for tocopherol concentration assays, and samples from ten different tissues were collected from slaughtered cows. Identification of α-tocopherol in tissues was confirmed by HPLC retention times and by comparison of mass spectra with that of α-tocopherol standards. The d-α-tocopherol and its acetate ester increased plasma tocopherol concentration faster than the racemic products with the greatest response occurring with d-α-tocopherol. Across all treatments, the highest α-tocopherol concentrations were noted in the adrenal gland and liver with the lowest found in muscle and thyroid tissue. Tissue analyses confirmed that in adrenal gland, kidney, liver and lung, α-tocopherol concentrations were higher following d-α than dl-α-tocopherol supplementation.

CHAPTER 6
PLASMA TOCOPHEROL IN RUMINANTS AFTER INGESTION OF FREE OR ACETYLATED TOCOPHEROL

Introduction

Administration of a massive dose of vitamin E is one of the usual methods in the prophylaxes of vitamin E deficiencies (Ogihara et al., 1985). Overman et al. (1954) reported in humans that a single massive oral dose of vitamin E is effective in increasing the free tocopherol plasma level to a significant degree after six hours. This temporary rise with ensuing drop has been designated as a tolerance curve. The tolerance curve mirrors the intestinal absorption of vitamin E. However, on this subject, the data for ruminant animals are sparse. Indeed, in ruminant species with their unique digestive system and physiologic differences in metabolic mechanisms, there are only few data on the bioavailability of the various forms of vitamin E. For this reason, the objective of the experiment was to gain information on blood plasma response to α-tocopherol in sheep or cattle provided with different chemical forms of tocopherols but with identical weights of these compounds, namely dl-α-tocopherol and dl-α-tocopheryl acetate.

Materials and Methods

Trial 1

Ten yearling crossbred wethers weighing 40 to 50 kg were used. All animals originated from a flock fed a commercial diet that consisted of corn meal, 56.45%, soybean meal (44% CP), 16.5%, cottonseed hulls, 25%, trace mineral salt, 1%, monocalcium phosphate, 1%, and .037% of vitamin A and D_3. They were continued on this diet throughout the experiment. Sheep were placed in individual metabolism cages 10 days before administrations of vitamin E as an adjustment period. They were then randomly assigned to two groups of five sheep each. Each animal received a single intraruminal dose of either (1) dl-α-tocopherol (1 mg = 1.10 IU) or (2) dl-α-tocopheryl acetate (1 mg = 1.0 IU) at a rate of 100 mg/kg body weight.

Trial 2

Four crossbred dairy heifers (averaging 250 kg) were used in a crossover design with two 20 d periods. They were given ad libitum access to hay and water during the study. In the first period, two heifers were administered orally with dl-α-tocopherol and two with dl-α-tocopheryl acetate. The single dose given for each component was 50 mg/kg body weight. The end of the first period was followed by a three-wk washing period. Then the treatment was reversed and the procedures were repeated in period 2.

Blood Samples

Blood samples were withdrawn from the jugular vein in heparinized vacutainers and immediately were centrifuged. They were collected before vitamin E administrations and at precise intervals (Figures 6.1 and 6.2). Plasma was separated, frozen and stored at -20°C until analyzed for α-tocopherol.

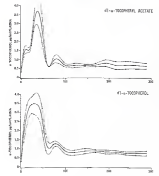

FIGURE 6.1. PLASMA TOCOPHEROL CONCENTRATION (µG/ML) IN SHEEP FOLLOWING ADMINISTRATION OF A SINGLE MEGADOSE (100 MG/KG BODY WEIGHT) OF (A) DL-α-TOCOPHERYL ACETATE OR (B) DL-α-TOCOPHEROL

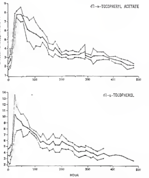

FIGURE 6.2. PLASMA TOCOPHEROL CONCENTRATION (µG/ML) IN CATTLE FOLLOWING ADMINISTRATION OF A SINGLE DOSE (50 MG/KG BODY WEIGHT) OF (A) DL-α-TOCOPHERYL ACETATE OR (B) DL-α TOCOPHEROL

Analytical Method

Plasma samples were prepared for high pressure liquid chromatography (HPLC) according to the method of McMurray and Blanchflower (1979). The chromatographic apparatus consisted of a model 6000 pump and WK septumless injector (Waters Associates, Milford, Mass.) A Perkin-Elmer 650-150 fluorescence spectrophotometer, equipped with a microflow cell unit, was used for quantification. Wavelength settings were 295 and 330 nm for excitation and emission, respectively. The column was a μ Bondapak C18 (3.9 mm x 30 cm) of 10 μm particle size purchased from Waters Associates (Milford, Mass.) The mobile phase, a solvent system (HPLC grade), consisted of methanol and water in a 97:3 ratio with a flow rate of 3 ml/minute.

Statistical Analysis

The indices of bioavailability were calculated for each individual sheep or cattle. These indices were: (1) the maximum α-tocopherol plasma (C max) concentration (peak of plasma α-tocopherol curves), (2) the time of maximum (t max) α-tocopherol concentration; and (3) the area (AUC) under the α-tocopherol plasma concentration time curve (Gibaldi and Perrier, 1975). Statistical analysis was carried out by analysis of variance (sheep) or covariance (cattle) by the general model procedure (SAS, 1980). The following model was used for the sheep,

$$Yij = \mu + t_i + E_{ij};$$

where μ = mean;

t_i = i^{th} type i = 1,2

E_{ij} = random error,

while for cattle the model used was,

$$Y_{ij} = \mu + C_0 + t_i + _{ij}$$

where C_0 = value at time 0.

Results

Trial I

Following the oral administration of the two vitamin E preparations to sheep, there was an increase in the plasma α-tocopherol level (Figure 6.1). The peak of this increase was reached faster ($P<.05$) in the dl-α-tocopherol than in its ester form (Table 6.1). The shorter T-maximum observed in the dl-α-tocopherol dosed group may have contributed to a faster onset of biological action. The data show that C_{maxima} plasma α-tocopherol concentrations had a tendency ($P>0.05$) to be higher in the dl-α-tocopherol than in the ester-dosed group. A direct comparison of the plasma tolerance curve area (AUC) between the two groups (Table 6.1), showed that the AUC was higher ($P<.05$) in the dl-α-tocopherol then in the sheep administered with dl-α-tocopheryl acetate (Table 6.1).

Maximum plasma vitamin E concentration, as well the tolerance curve, are dependent chiefly on factors effecting the absorption of the vitamin from the intestine. Our results showed that the effectiveness of the absorption indicated by the tolerance curve area was higher in the sheep dosed with free alcohol form than with the acetylated group. The higher peak observed in the plasma tolerance curve in the dl-α-tocopherol group than in its acetate form probably reflects its higher potency. At the end of the two-wk experimental

TABLE 6.1. DL-α-TOCOPHEROL PHARMACOKINETIC PLASMA VALUES (LEAST SQUARE MEANS ± SE) IN SHEEP AFTER A SINGLE ORAL ADMINISTRATION OF VITAMIN E (100 mg/kg BODY WEIGHT)

Cmax[1] (μg/ml)	Tmax[2] (hr)	Co[3] (μg/ml)	CmaxCo (μg/ml)	Ct[4] (μg/ml)	Ct/Co (μg/ml)	Cmax/Ct (μg/ml)	AUC[5] (μg/ml/hr)
				dl-α-tocopherol			
3.58±.22	26.5±4.04	.42±.06	8.99±1.13	.73±.07	1.80±.25	4.95±.54	332.18±20.27
				dl-α-tocopheryl acetate			
3.05±.20	40.4±3.61	.41±.05	7.67±1.01	.59±.06	1.50±.22	5.36±.48	269.63±18.13
				Significance (P)			
	<.05						<.05

---

[1] Maximum plasma concentration.

[2] Maximum time (hr).

[3] Initial plasma concentration.

[4] Terminal plasma concentration.

[5] Area under plasma concentration -- time curve.

[6] Significance tested following analysis of variance.

TABLE 6.2. TRIAL 2. D-α-TOCOPHEROL PHARMACOKINETIC PLASMA VALUES (LEAST SQUARE MEANS ± SE) IN CATTLE AFTER A SINGLE ORAL ADMINISTRATION OF VITAMIN E (50 mg/kg BODY WEIGHT).

Vitamin E Preparations	Cmax[1] (µg/ml)	Tmax[2] (hr-1)	Cmax/Co (µg/ml)	CT[3] (µg/ml)	CT (µg/ml)	Cmax/CT (µg/ml)	AUC[4] (µ/ml/hr-1)
dl-α-tocopherol	10.84	35.38	3.88	3.71	1.36	3.04	1995
dl-α-tocopheryl acetate	9.23	48.61	3.43	3.21	1.23	2.84	1589
SE	0.61	14.89	0.37	0.40	0.43	.39	88
Significance (P)							<.05

[1] Maximum plasma concentration.

[2] Maximum time (hr).

[3] Terminal plasma concentration

[4] Area under plasma concentration -- time curve

period, the terminal plasma tocopherol levels in both groups were higher ($P<.05$) than the original values.

Trial 2

A few hours after oral dosing in both groups of cattle there was an increase in plasma α-tocopherol levels (Figure 6.2). The free form was absorbed with higher effectiveness than its ester form. This is shown by its greater plasma tolerance curve area and its tendency to higher maxima concentration and speedier t maxima appearance. There was significantly less utilization of the ester form versus the alcohol form. In the present experiment, the system of rotation used in administering the two vitamin E doses minimized the individual variations.

Discussion

In the present experiment, the plasma persistence curve was mainly used to provide a criterion of availability of vitamin E for physiological function following oral dosage to ruminants with two isomeric forms.

The present results show that in the ruminal environment, despite a strong reducing system, the dl-α-tocopherol was preserved sufficiently to induce a superior persistence curve area response to that of identical weight of the esterified form. It is possible that the degree of hydrolysis could be an important factor in the shape of the tolerance curve. As the stability of free tocopherol is known to be inferior to that of tocopherol esters, it may be hypothesized that the relative less favorable response to tocopherol ester is due to factors influencing the hydrolysis of these esters.

It seems probable that in cattle, like other animals, dl-α-tocopheryl acetate following hydrolysis is split in the intestinal mucosa and is absorbed as free tocopherol from the gut and passed to the systemic circulation via the lymphatic system. According to Tikriti (1969), in cattle the acetylated form of vitamin E is hydrolyzed in the digestive system, beginning in the rumen, and appears to be utilized somewhat less efficiently than the free form. One cannot rule out, as it was suggested by Gallo Torres et al. (1971), the possibility of a rate-limiting hydrolytic reaction occurring in the lumen of the intestine prior to entrance of the vitamin E into the intestinal wall of the ruminant animal.

Our results showed that T-maxima is much longer in ruminants than in humans following oral loading with vitamin E. Indeed, Hashim and Schattinger (1966) observed that in normal human subjects plasma tocopherol levels began to rise between 2 and 4 h and peaked at 7.5 h following oral administration of vitamin E. They also observed that at 24 h the plasma tocopherol level had declined and that at 48 h post absorptive levels were reached. Marusich et al. (1967) reported that in chickens, following the administration of a single oral dose (50 IU of dl-α-tocopheryl acetate), the peak plasma values obtained at 6 h were followed by a steady decline. However, in rats, Marusich et al. (1968) observed that plasma tocopherol levels peaked at 24 h, following administration of a single oral dose of dl-α-tocopheryl acetate.

The fact that the biological response to dl-α tocopherol was greater than with its acetylated form agrees with the results reported in humans (Baker et al. 1980). These workers observed that following administration of a single oral dose (800 mg) of dl-α-tocopheryl acetate, plasma d-α-tocopherol peaked (50% from the initial value) 8 h after ingestion. The same dose of free tocopherol induced a greater rise (60% from the original value) in 5 h.

It seems that dl-α-tocopherol serves more pharmacological action when ingested in amounts many times the generally recognized nutritional requirements than its esterified form. The results demonstrated that T max was faster following oral dosing of the free than acetylated form. This suggests that the hydrolysis rate of esterified tocopherol to the physiologically active free tocopherol is quite slow.

In this study, the area (AUC) under the α-tocopherol plasma concentration time curve undoubtedly was influenced by the amount of vitamin E activity again. Indeed, the higher vitamin E potency of dl-α-tocopherol (1.1 IU/mg) compared to 1.0 IU/mg, for the acetate form probably contributed to the tendency of a higher plasma vitamin E peak as well as the greater AUC.

In the present experiment, initial plasma tocopherol concentrations (2 to 3 µg/ml) from hay-fed cows were in the range of values observed by Pehrson and Hakkarainen (1986) and Hakkarainen et al. (1987). The initial value of α-tocopherol in the plasma of sheep fed a commercial diet was very low. In grain-fed sheep, Caravagi (1969) reported plasma concentrations of .89 ± .16 SD µg/ml. Storer

(1974) observed higher plasma tocopherol concentrations (1.28 ± [illegible] µg/ml) for sheep fed dry feed. It appears that in the ruminant animal, following a single oral dose of dl-α-tocopherol or its acetylated form, the terminal values were higher in sheep and cattle than the initial values. This may be related to a slow saturation of tissues with both forms of tocopherol. However, no difference was observed in the elimination rates (C max/CT). In conclusion, the data in ruminants confirm the higher biological potency of the dl-α-tocopherol vs. dl-α-tocopheryl acetate

Summary

Two trials were carried out in order to evaluate the bioavailability of dl-α-tocopherol and dl-α-tocopheryl acetate administered to sheep or cattle in a single oral dose. In the first trial, two groups of five sheep were used. They received 100 mg/kg body weight of either dl-α-tocopherol or its acetylated form. The blood plasma tolerance curve area and the maximum plasma concentration were higher in the dl-α-tocopherol group than in its ester form. The time (h) for maximum plasma α-tocopherol concentration was quicker ($P<0.05$) in the dl-α-tocopherol group than in its ester form. In a second trial, four heifers received the two forms (50 mg/kg body weight) in rotation after an appropriate washing period between the two dosings. Again, a greater plasma tolerance curve area was observed in the cattle following administration of dl-α-tocopherol than its acetylated form.

CHAPTER 7

PLASMA AND TISSUE VITAMIN E CONCENTRATIONS IN SHEEP AFTER ADMINISTRATION OF A SINGLE INTRAPERITONEAL DOSE OF dl-α-TOCOPHEROL

Introduction

There have been remarkably few systematic investigations on the disposition of pharmacologic doses of vitamin E in sheep. Massive doses in sheep can be given orally, intramuscularly (IM) or intravenously (IV) (Hidiroglou and Karpinski, 1987). Bioavailability of vitamin E after intramuscular (IM) administration has recently been reported (Hidiroglou and McDowell, 1987).

A potential alternative to IV or IM dosing could be the delivery of vitamin E into the peritoneal space. Since no studies have been published on the disposition kinetics of vitamin E in sheep plasma and tissues following administration of a single intraperitoneal (IP) dose of vitamin E, such a study using dl-α-tocopherol was undertaken.

Experimental Procedure

Animals

Twenty-five clinically normal, one-year-old, crossbred wethers were used in the study. The sheep weighed from 30 to 35 kg at the beginning of the experiment. A standard diet (Table 7.1) and water were available ad libitum. Five sheep were selected at random and slaughtered at d 0 as the control with no treatment. Thereafter, 4

TABLE 7.1. DIET OF SHEEP[a]

Ingredient	(%)
Corn meal	56.45
Soybean meal	16.50
Cottonseed hulls	25.00
Trace mineral salt[b]	1.00
Dicalcium, monocalcium phosphate	1.00
Vitamin A, D_3[c]	0.037

[a] Analyzed for vitamin E and Se (8 ppm and .235 ppm, respectively)

[b] Provided, per kilogram of diet: 2.1 g NaCl, .22 mg I, .068 mg Co, 9 mg Fe, .725 mg Cu, 2.625 mg Mn and 8.75 mg Zn.

[c] Provided, per kilogram of diet: 5,000 IU vitamin A and 500 IU vitamin D.

sheep were selected at random and killed at each of days 3, 6, 10, 15 and 28 after dosing.

Five g of dl-α-tocopherol were given intraperitoneally (IP) to each treated sheep as a single injection in emulsion (Tween 80) with a final volume of 75 ml. The IP dose was administered using the technique of Hurter (1987). Blood samples were withdrawn by jugular puncture at designated times prior to (0) and after vitamin E administration (3, 6, 10, 15 and 28 d) and collected in heparinized tubes. Plasma was obtained upon centrifugation at 500 x g for 15 min. The animals were killed by exsanguination. Portions of kidney, liver, adrenal, pancreas, skeletal muscles, heart, spleen and lung were removed. All tissues were rinsed in water and their surfaces dried with filter-paper. During removal of all tissues, care was taken to dissect away superfluous adipose tissue. All tissues and plasma were stored at -20°C until analyzed for α-tocopherol concentration.

Analytical Methods

Quantitation of d-α-tocopherol in blood plasma and tissues was performed by high pressure liquid chromatography (HPLC) using a fluorescent detector (McMurray and Bleachflower, 1979). The HPLC consisted of an M6000 pump and WK septumless injector. A Perkin-Elmer 650-10S fluorescence spectrophotometer equipped with a microflow-cell unit was used for quantification. Wavelength settings were 295 and 330 nm for excitation and emission, respectively. The column was a μ Bondapak C_{18} (3.9 mm x 30 cm) of 10 μm particle size. Elution was performed with methanol:water (97:3) solvent with a flow rate of 3 ml/min. All solvents used for the HPLC mobile phase or for the extraction were HPLC or pesticide grade. Tissue samples for vitamin E determination were prepared according to the method of Bartos et al (1985).

Statistics

One-way analysis of variance (ANOVA) was performed on the logarithms of the tissue values and the tissue to plasma ratios. Logarithms were taken to stabilize the variances which increased with increasing concentrations. To further study the effect of time, five contrasts were examined (SAS Institute, 1985). Untransformed means have been presented with approximate standard errors based on the analysis of the logarithms. Residuals were examined and an outlier was identified using Tietjen and Moore's statistic (Tietjen and Moore, 1972). The changes which occurred when this outlier was removed from the analysis are noted.

Plasma values were also analyzed by analysis of variance. This analysis was a one-way ANOVA of time within the animal. Here also, a transformation to logarithms was necessary and the time effect was examined by several contrasts.

Results

Mean levels of dl-α-tocopherol in tissues and plasma at the time of slaughter are given in Table 7.2. The accompanying analysis of variance of the logarithms of these values at slaughter is given in

TABLE 7.2. MEAN LEVELS OF α-TOCOPHEROL IN TISSUES (μg/g FRESH) AND PLASMA (μg/ml) AT TIME OF SLAUGHTER.

	Time (day)								
	0	3		6	10	15	28	SEM[a]	
Kidney	0.96	28.07	(33.09)[b]	8.25	6.80	5.46	3.96	.21M	(.19M)
Liver	0.97	212.61	(270.01)	69.26	37.20	34.42	15.71	.26M	(.17M)
Adrenal	1.70	42.25	(47.67)	24.59	35.51	20.21	33.86	.21M	(.20M)
Pancreas	11.36	28.50	(31.59)	23.19	24.40	20.74	28.60	.11M	(.12M)
Neck	1.47	7.65	(9.24)	4.54	4.57	5.24	4.49	.25M	(.23M)
Leg	1.56	7.55	(9.38)	4.59	4.52	6.63	3.96	.25M	(.19M)
Heart	2.90	23.55	(27.82)	16.84	16.40	14.73	11.03	.18M	(.16M)
Spleen	2.73	125.20	(160.60)	35.12	27.93	25.13	9.74	.35M	(.27M)
Lung	2.09	164.85	(216.34)	128.75	102.66	33.22	12.19	.43M	(.25M)
Plasma	0.56	5.43	(5.97)	3.13	2.23	2.22	1.96	.14M	(.14M)

[a] The standard error of the mean is based on the analysis of variance on the transformed scale and is approximate. It varies with the mean, M, and has been expressed in terms of it.

[b] The values in parentheses are the results when one outlier was removed.

Table 7.3. There was a significant time effect for all the tissues. In most tissues, the tocopherol concentrations recorded at 3 d after dosing were the largest, but the various tissues responded differently to vitamin E injection. Examination of the tissue data 3 d postdosing revealed a striking accumulation of injected vitamin E in the liver. The lungs had the next highest concentration at that date. The spleen invariably contained a considerable amount of α-tocopherol 3 d post initiation. α-Tocopherol concentrations in the remaining tissues, also 3 d after dosing, showed a considerable increase but to a smaller degree. The contrasts over time give some indication of when a plateau had been reached, but it must be kept in mind that significance may fail to be achieved because of large variances or insufficient data. For example, for the kidney the mean of days 15 to 28 is not different ($P > .05$) from the mean at day 10. This indicates there is no further significant change from the level reached by day 6, suggesting a plateau has been reached. However, as the means continue to decrease up to and including day 28, it would seem inappropriate to draw this conclusion. Similarly, the significance tests suggest the neck, leg, heart, adrenal and pancreas have reached a plateau by 3 d. The liver and lung do not appear to have reached a plateau by 15 d, however. The contrasts suggest the spleen has reached a plateau by day 10, although the mean decreases on d 28.

There were particularly low values recorded for one animal at d 3. Tietjen and Moore (1972) statistics indicated this value was an outlier ($P < .05$) for liver, spleen and lung and it was an extreme

TABLE 7.1. ANALYSIS OF VARIANCE MEAN SQUARES OF THE LOG TISSUE VALUES

	Source of variation						
Tissue	Overall time effect	3-28[a] versus 0	6-28 versus 3	10-28 versus 6	15-28 versus 10	28 versus 15	Residual
DOF	5	1	1	1	1	1	19
Kidney	5.036**	17.612**	6.424**	0.687*	0.286[b]	0.171	0.152
Liver	14.382**	59.183**	8.517**	2.350**	0.566(*)	1.294*(**)	0.235
Neck	1.413**	6.709**	0.312(*)	0.019	0.004	0.019	0.212
Leg	1.527**	6.907**	0.187(*)	0.009	0.028	0.503	0.210
Heart	2.466**	11.353**	0.484(**)	0.162	0.238	0.091	0.117
Spleen	7.057**	24.121**	7.422**	1.368(*)	1.276(*)	1.097	0.413
Lung	11.984**	43.916**	2.144(**)	3.898*(**)	7.572**	2.388(**)	0.574
Adrenal	4.522**	20.818**	0.647(*)	0.086	0.398	0.658(*)	0.149[c]
Pancreas	0.468**	2.063**	0.083	0.003	0.000	0.169	0.063[c]

[a] Mean of treatments 3, 6, 10, 15 and 28 (3-28) are compared to control group 0.

[b] Significance levels in parentheses note changes which occurred when one outlier was removed.

[c] Residual is based on 17 degrees of freedom because of missing observations.

* (**)Indicates a significant effect at 5% (1%) level

observation for several other variables. The effect it has on the results is noted in the tables. The main effect of its omission was a decrease in the variances and an increase in the values at d 3. The result is, the contrast tests shift the plateau to d 6 for the neck, leg, heart and adrenal which seems quite reasonable studying the means. As well, the increase at d 28 becomes significant (P<.05) for the adrenal. At 28 d, the skeletal muscles and kidney had the lowest values among the various tissues, while the adrenal ranked first followed by the pancreas.

The means for the ratio of the tissue to plasma (t/p) levels are given in Table 7.4 and the associated analysis of variance in Table 7.5. The observations at d 3 did not fail the Tietjen and Moore test (P>.05) for any variable. However, its effect has been noted in the tables. Here there is no effect (P>.05) over time for the neck and heart. While the overall test is not significant (P>.05), the contrast of d 3 to later times indicates d 3 values were depressed for these two tissues. The leg data show a similar pattern although the overall time effect was significant in this case. After a significant increase, the kidney data appear to achieve a plateau by d 6, the spleen by d 10. Liver and lung data also show a significant increase and then a decrease (P<.01). Although the tests indicate a plateau is reached, the means suggest that a decrease may continue to d 28. The adrenal and pancreas data show a significant time effect, but without a plateau.

In Table 7.6, summary statistics are given for the four animals whose plasma values were recorded throughout the experiment. These

TABLE [illegible]. MEANS OF THE RATIO OF TISSUE TO PLASMA LEVELS OF α-TOCOPHEROL AT TIME OF SLAUGHTER

	Time (day)								
		3		6	10	15	28	SEM[a]	
Kidney	1.82	5.18	(5.78)	2.65	3.08	2.56	2.04	.20M	(.20M)[b]
Liver	1.97	36.84	(45.60)	21.80	17.02	16.57	8.26	.25M	(.21M)
Adrenal	3.72	7.95	(8.34)	7.84	16.15	9.89	17.00	.24M	(.25M)
Pancreas	18.36	5.29	(5.37)	7.73	10.94	9.74	14.62	.12M	(.13M)
Neck	2.74	1.37	(1.58)	1.42	2.09	2.35	2.32	.21M	(.21M)
Leg	2.81	1.36	(1.63)	1.48	2.08	3.09	2.03	.21M	(.18M)
Heart	6.09	4.24	(4.73)	5.43	7.40	6.35	5.60	.17M	(.17M)
Spleen	5.13	21.50	(27.01)	11.16	12.53	11.16	4.96	.30M	(.24M)
Lung	4.54	29.29	(36.15)	42.54	47.52	15.82	6.07	.43M	(.31M)

---

[a] The standard error of the mean is based on the analysis of variance on the transformed scale and is approximate. It varies with the mean, M, and has been expressed in terms of it.

[b] The values in parentheses are the results when one outlier was removed.

TABLE 7.5. ANALYSIS OF VARIANCE MEAN SQUARES OF THE [illegible] PLASMA.

Tissue	Overall time effect	3–28^a versus 0	6–28 versus 3	10–28 versus 6	15–28 versus 10	28 versus 15	Residual
DOF	5	1	1	1	1	1	19
Kidney	0.432*	0.765*	1.128*(**)^b	0.021	0.181	0.064	0.138
Liver	4.657**	19.106**	1.090**	0.719(*)	0.414	0.955(*)	0.222
Neck	0.415	0.535	0.836*(NS)	0.676	0.029	0.001	0.165
Leg	0.510*(NS)	0.481	1.081*(NS)	0.611(*)	0.076	0.301	0.153
Heart	0.170	0.002	0.604*(NS)	0.080	0.144	0.020	0.109
Spleen	1.231*(**)	2.527*(**)	1.567*(**)	0.235	1.042(*)	0.787	0.310
Lung	4.298**	10.924**	0.000	1.663(*)	6.985**	1.919(**)	0.577
Adrenal	1.189**	3.323**	0.447	0.958*	0.273	0.943*	0.202^a
Pancreas	0.739**	1.520**	1.416**	0.368*	0.012	0.326*	0.058^a

^{a}Residual is based on 17 degrees of freedom because of missing observations.

^{b}Significance levels in parentheses note changes which occurred when one outlier was removed.

* (**)Indicates a significant effect at 5% (1%) level. NS indicates non-significance at 5% level.

TABLE 7.6. PLASMA TOCOPHEROL (μg/ml) VALUES AND SUMMARIES FOR THOSE ANIMALS SLAUGHTERED AT 28 DAYS.

Days	Animal 1	2	3	4	Mean	SEM
Day 0	0.58	0.47	0.78	0.48	0.58	0.072
Day 1	6.41	6.09	7.27	3.59	5.84	0.790
Day 2	2.89	3.67	6.64	6.56	4.94	0.972
Day 3	1.95	2.81	4.53	8.13	4.36	1.368
Day 6	1.64	2.34	3.59	2.66	2.56	0.405
Day 10	1.56	1.72	1.87	1.44	1.65	0.094
Day 15	1.44	1.15	–[a]	1.64	1.41	0.142
Day 28	1.88	1.64	2.03	2.27	1.96	0.132
Area[b]	51.43	52.56	74.77	71.96	62.68	6.199
Maximum concentration plasma (μg/ml)	6.41	6.09	7.27	8.13	6.98	0.459
Time to maximum concentration (d)	1.00	1.00	1.00	3.00	1.50	0.500
Elimination[c] rate	3.41	3.71	3.58	3.58	3.57	0.062

[a] No measurement was taken at this point in time.

[b] Total area under the plasma concentration curve.

[c] Elimination rate is defined as the maximum/terminal plasma level.

values were typical of those recorded for all the [illegible] up to the time of their slaughter

Discussion

Liver and spleen were characterised by a high accumulation of vitamin E. Similar results were observed in the liver and spleen of rats following IP dosing with a large amount of vitamin E (Peake and Bieri, 1971). This route of administration did not cause any alteration in the metabolism of vitamin E. Indeed, Black and Bieri (1964) demonstrated that following IP dosing of rats with ^{14}C-d-a-tocopherol the radioactivity recovered in the livers was an unchanged a-tocopherol. There is an increase in the intensity of exchange by the tissues, metabolism and elimination from the body when this vitamin is given to sheep IP.

The important uptake of vitamin E by the sheep liver supports the concept that the liver is a target for vitamin E action. Vitamin E is delivered to the liver via the portal vein and returned to the rest of the body by the hepatic vein. According to Kayden and Traber (1987), there must be mechanisms in the liver for the regulation of tocopherol incorporation into lipoproteins, its storage in the liver and for its excretion. When a massive dose of vitamin E was given to sheep IP, there was a peak 3 d post dosing in the vitamin E concentration in all tissues and then a decline. The greatest rate of loss occurred in the hepatic tissue, where the loss was proportional to its concentration in vitamin E. Bieri (1972) reported that all tissues (except depot rat contains a labile pool of α-tocopherol which mobilizes rapidly, and a fixed component which is retained for long periods.

The high concentrations of vitamin E in the liver, spleen and lung, 3 d after initiation of the IP administration to sheep are in agreement with the results of Gallo-Torres (1971) in rats where these organs contained high levels of vitamin E following parenteral administration of this vitamin. In the lung very high concentrations of vitamin E could be attributed to this organ's abundant blood supply. The high tissue/plasma (t/p) ratio observed in the adrenal and pancreas of sheep provided with IP vitamin E characterizes the intensity of metabolism in these organs. Tikriti et al. (1968) observed in dairy cows that, following parenteral administration of radiotocopherol, pancreas and adrenal were relatively high in vitamin E activity. The heart t/p indicated an intermediate vitamin E activity while in the muscle the t/p was low. This might indicate that the muscle has a different mechanism of action concerning handling of vitamin E than other organs.

Gallo-Torres (1982) noted that information on the fate of vitamin E after parenteral administration is scarce. The present work provided data on the fate of a massive single IP dose of vitamin E in the body of sheep, indicating a differential handling by the various organs. Judging from the results of vitamin E distribution it appears that the IP method is a convenient route of vitamin E administration to sheep.

Summary

Blood plasma and tissue vitamin E were determined in twenty five crossbred sheep following intraperitoneal injection of dl-α-tocopherol. Five sheep were used as controls (no treatment and killed at d 0). The remaining 20 sheep were administered intraperitoneally with 5 g of dl-α-tocopherol. From these twenty vitamin E dosed sheep, four were slaughtered at each of d 3, 6, 10, 15 and 28 after dosing. There was a significant time effect for all α-tocopherol concentrations for all tissues. In meat tissues, the peak for α-tocopherol concentrations was observed at 3 d post dosing. There was a varied rate of uptake for different tissues examined. Three d post IP dosing, a large uptake of vitamin E by the liver was noticed, and this supports the concept of the hepatic tissues as a target organ for vitamin E action. Also at d 3, a pronounced uptake was observed in the spleen and lungs. Vitamin E concentrations in the remaining tissues at d 3 post dosing showed also a considerable increase, but to a lesser degree than those in liver, spleen and lung. A decline of vitamin E concentration in all tissues occurred after d 3. This study, through the use of contrasts over time (d), provided some indication of when a plateau in vitamin E concentrations had been reached for each individual tissue following an IP dosing.

CHAPTER 8
GENERAL CONCLUSIONS

Five experiments were carried out to investigate the bioavailability of various tocopherol sources in ruminants as well as the effect of mode of administration. Experiment I was conducted to investigate the bioavailability of d-α-tocopherol and dl-α-tocopherol suspended in sesame oil solution (200 mg/ml) and administered intramuscularly at a rate of 40 mg/kg body weight. In all sheep given the various vitamin E sources by intramuscular oil depot injection, a delayed increase in plasma α-tocopherol levels occurred. Sheep administered d-α-tocopherol and slaughtered after 360 hr had a larger (P<.01) area under the plasma concentration time curve (AUC) than sheep injected with d-α-tocopherol or dl-α-tocopherol and killed 240 hr post dosing. A higher plasma C maxima (P<.01) was found in the d-α-tocopherol group than in the others. Pancreatic, hepatic, lung, spleen and muscle α-tocopherol concentrations were higher (P<.05) for this group than for controls. Tendencies for higher (P>.05) tissue α-tocopherol concentrations were found for the d-α-tocopherol administered group (360 hr) than in the other vitamin E treated sheep.

Experiment II assessed the bioavailability of four tocopherol sources provided orally of either (1) dl-α-tocopherol, (2) dl-α-tocopheryl acetate, (3) d-α-tocopherol or (4) d-α-tocopheryl acetate in crossbred yearling wethers at a level of 400 IU/day/sheep for 28 d. Higher α-tocopherol concentrations (P<.01) were found in the heart and lung of the sheep provided with d-α-tocopherol than the other forms. In all diets, pancreas contained the highest concentration followed by the liver and spleen. Muscle and adipose tissue were found to have the lowest ($P<.05$) concentration of α-tocopherol. Sheep provided d-α-tocopherol had a larger ($P<.05$) area under the plasma α-tocopherol concentration time curve than the other groups. Following the provision of 400 IU's/d of the various tocopherol sources to sheep, it was found that d-α-tocopherol was more potent than its acetate or the l-isomeric forms of vitamin E.

In experiment III beef cows were provided a daily oral dose of 1000 IU of various tocopherol sources for 28 d to investigate plasma and tissue α-tocopherol concentrations. Supplementation over a 1 to 7 d period of time with the various vitamin E preparations increased ($P<.01$) plasma α-tocopherol concentration above baseline values. d-α-tocopherol and its corresponding acetate ester increased plasma α-tocopherol concentration quicker ($P<.05$) than the racemic products with the greatest response observed with d-α-tocopherol. There was a wide distribution of α-tocopherol concentration in the tissues. Across all treatments, the highest α-tocopherol concentrations were found in the adrenal gland and liver with the lowest observed in muscle and thyroid tissue. Higher α-tocopherol concentrations were found in the adrenal gland, kidney ($P<.05$), liver and lung following d-α- than dl-α-tocopherol supplementation

In experiment IV, dl-α-tocopherol and its ester were administered to sheep intraruminally (100 mg/kg b.w.) or cattle (50 mg/kg b.w.) in a single oral dose. The sheep data showed that the C maxima plasma α-tocopherol concentration had a tendency [illegible] to be higher in the dl-α-tocopherol than in the ester-dosed group. A higher (P<.05) plasma α-tocopherol tolerance curve area was found in the dl-α-tocopherol than in its ester form, as well as a quicker (P<.05) time (h) for maximum plasma α-tocopherol concentration. Data from cows revealed a greater (P<.05) plasma tolerance area following the administration of dl-α tocopherol than its acetylated form.

Experiment V was designed to determine plasma and tissue α-tocopherol concentrations in sheep following a 5 g dose of dl-α-tocopherol (water based) injected intraperitoneally followed by serial slaughterings. A significant time effect for all tissues was noticed while the peak for α-tocopherol concentrations was observed 3 d post dosing. A varied rate of uptake of α-tocopherol was noticed with the liver, lung and spleen showing a pronounced uptake while the muscle showed the least. This study provided an indication of when a plateau in α-tocopherol concentration had been reached for each individual tissue. Studies reported in this dissertation indicated that blood plasma and tissue α-tocopherol concentrations were influenced by various sources of vitamin E as well as by mode of administration and its type of vehicle. Higher indexes of bioavailability were observed in favor of natural α-tocopherol than synthetic α-tocopherol in sheep and cattle dosed with equivalent amounts (IU). Sheep given the vitamin E preparations by intramuscular oil depot injection resulted in a delayed increase in plasma and tissue α-tocopherol concentration.

Intraperitoneal injection of dl-α-tocopherol (water base) resulted in a substantial and quick rise in plasma and tissue α-tocopherol levels. Comparing the currently accepted biological potencies (IU/mg) provided by the National Formulary (1985) for the various tocopherol sources to the estimate values for ruminants are as follows respectively: d-α-tocopherol (1.49, 3.60), d-α-tocopheryl acetate (1.36, 2.58), dl-α-tocopherol (1.1, 1.37). The results obtained from these experiments suggest that the current accepted biological potencies of the various tocopherol sources may be invalid for ruminants.

Experiments reported in this dissertation show that the natural form of α-tocopherol had a higher bioavailability than did the synthetic, though the reason for this remains unresolved.

Further research in ruminants should be directed towards identifying the potential presence of specific binding proteins in tissues of ruminants which have been shown to be fairly specific for the natural stereoisomer in other species.

LITERATURE CITED

Adamstone, F. B. [illegible] Histologic comparison of brains of vitamin A deficient vitamin E deficient chicks. Arch. Pathol. 43:301

Alaupovic, P., B. C. Johnson, Q. Crider, H. N. Bhagavan, and B. J. Johnson. 1961. Metabolism of alpha-tocopherol and the isolation of non-tocopherol-reducing substance from animal tissues. Am. J. Clin. Nutr. 9:76.

Alderson, N. E., G. E. Mitchell, Jr., C. O. Little, R.L. Warner, and R. E. Tucker. 1971. Preintestinal disappearance of vitamin E in ruminants. J. Nutr. 101:655.

Ames, R. S. 1971. Isomers of alpha tocopheryl acetate and their biological activity. Lipids 6:281.

Ames, R. S. 1979. Biopotencies in rats of several forms of alpha-tocopherol. J. Nutr. Res. 109:2198.

Ames, R. S., M. J. Ludwig, D. R. Nelan, and C. D. Robeson. 1963. Biological activity of an l-epimer of d-alpha-tocopheryl acetate. Biochemistry 2:188

Baker, H., O. Frank, B. Deangelis, and S. Feingold. 1980. Plasma tocopherol in man at various times after ingesting free or acetylated tocopherol. Nutr. Rep. Int. 21:531

Baker, H., G. J. Handelman, S. Short, L. J. Machlin, H. N. Bhagavan, E. A. Dratz, and O. Frank. 1986. Comparison of plasma alpha tocopherol and gamma-tocopherol levels following chronic oral administration of either all-rac-alpha-tocopheryl acetate or RRR-alpha-tocopheryl acetate in normal adult male subjects. Am. J. Clin. Nutr. 43:382

Balard, B. E. 1968. Biopharmaceutical considerations in subcutaneous and intramuscular drug administration. J. Pharm. Sci. 57:357.

Behrens, H., G. Matschullat, and K. Tuck. 1975. Uber die vertraglichkeit einiger vitaminlosungen beim schaf nach intramuskularer applikation. Dtsch. Tierarzt. Wschr. 82:1

Behrens, W. A., J. N. Thompson, and R. Madere. 1982. Distribution of alpha-tocopherol in human plasma lipoproteins. Am. J. Clin. Nutr. 35:691.

Bieri, J. G. 1972. Kinetics of tissue α-tocopherol depletion and repletion. Ann. N. Y. Acad. Sci. 203:181.

Bieri, J. G., L. Corash, and V. S. Hubbard. 1983. Medical use of vitamin E. New Engl. J. Med. 308:1063.

Bieri, J. G., and E. L. Prival. 1967. Vitamin E activity and metabolism of N-methyl tocopheramines. Biochem. J. 6:2153.

Bille, N., J. Carstensen, E. Leerbeck, and H. Sondergaard. 1976. Persistence of intramuscular deposits of vitamin D_3 in slaughter pigs. Nord. Vet. Med. 28:496.

Black, P. A., and J. G. Bieri. 1964. Metabolic products of α-tocopherol in the livers of rats given intraperitoneal injections of ^{14}C-α-tocopherol. Biochimica et Biophysica 84:729.

Blaxter, K. L., and F. Brown. 1952. Vitamin E in the nutrition of farm animals. Nutr. Abstr. Rev. 22:1.

Blaxter, K. L., and G. A. M. Sharman. 1953. Prevention and cure of enzootic muscular dystrophy in beef cattle. Nature 172:1006.

Bliss, C. I., and P. Gyorgy. 1967. The Vitamins, Vol. VI. Vitamin E Assay by Chemical Methods, pp. 304-316. Academic Press, New York.

Blomstrand, R., and L. Forsgren. 1968. Labelled tocopherols in man. Int. Z. Vitaminforsch. 38:328.

Bolliger, H. R., and M. L. Bolliger-Quaife. 1956. Intern. Kongress Vitamin E 3, Venedig 1955, 30.

Bonetti, E., and F. Stirpe. 1963. Effect of selenium on muscular dystrophy in vitamin E deficient rats and guinea pigs. Proc. Soc. Exptl. Biol. Med. 114:109.

Boyer, P. D. 1960. Vitamin E. The Enzymes. Prosthetic Groups and Cofactors (Part B) (P. D. Boyer, H. Lardy, and K. Myrbach, eds.), Rev. Vol. 3, pp. 353-368. Academic Press, New York.

Brubacher, G., and H. Weiser. 1968. Tocopherol. Wiss. Veroeff. dtsch. Ges. Ernaehrung, Hrsg (K. Lang, ed.), Vol. 16, p. 50. Steinkopff-Verlag Darmstadt.

Bunyan, J., A. T. Diplock, E. E. Edwin, and J. Green. 1962. Exudative diathesis and lipid peroxidation in the chick. Brit. J. Nutr. 16:519.

Bunyan, J., J. Green, E. A. Murrell, A. T. Diplock, and M. A. Cawthorne. 1968. On the postulated peroxidation of unsaturated lipids in the tissues of vitamin E deficient rats. Brit. J. Nutr. 22:97.

Burton, G. W. 1989. Antioxidant action of [illegible]. J. Nutr. 119:109.

Burton, G. W., A. Webb, and K. U. Ingold. 1985. A mild, rapid, and efficient method of lipid extraction for use in determining vitamin E/lipid ratios. Lipids 20:29.

Caravaggi, C. 1969. Vitamin E concentrations in the serum of various experimental animals. Comp. Biochem. Physiol. 30:585.

Caravaggi, C., M. W. Gibbons, and F. Wright. 1968. The appearance of tocopherol in the blood of sheep after intramuscular injection of α-tocopheryl acetate. N.Z. J. Agric. Res. 11:313.

Catignani, G. L., and J. G. Bieri. 1977. Rat liver α-tocopherol binding protein. Biochim. Biophys. Acta 497:349.

Cheeseman, K. H., G. W. Burton, K. U. Ingold, and T. F. Slater. 1984. Lipid peroxidation and lipid antioxidants in normal and tumor cells. Toxicol. Pathol. 12:235.

Chernick, S. S., J. G. Moe, G. P. Rodnan, and K. Schwarz. 1955. A metabolic lesion in dietary necrotic liver degeneration. J. Biol. Chem. 217:829.

Chow, C. K. 1979. Nutritional influence on cellular antioxidant defense systems. Amer. J. Clin. Nutr. 32:1066.

Chow, C. K., H. H. Draper, A. S. Csallany, and H. Chiu. 1967. The metabolism of ^{14}C alpha-tocopheryl quinone and ^{14}C alpha-tocopheryl-hydroquinone. Lipid 2:390.

Corwin, L. M., and K. Schwarz. 1960. Maintenance of α ketoglutarate and succinate oxidation in E-deficient liver homogenates by alpha-tocopherol, a tocopherol metabolite, menadione and diphenylphenylenediamine. Nature (London) 186:1048.

Csallany, A. S., and H. H. Draper. 1963. The structure of a dimeric metabolite of D-alpha tocopherol isolated from mammalian liver. J. Biol. Chem. 238:2912.

Csallany, A. S., and H. H. Draper. 1964. Cis-trans-isomers of dl-alpha tocopherone. J. Biol. Chem. 239:574.

Corte, G. M. 1966. Fat soluble vitamins in veterinary science. Riv. Zootec. 39:200.

Dam, H. 1944. Studies on vitamin E deficiency in chicks. J. Nutr. 27:193.

Dam, H. 1957. Influence of antioxidants and redox substances on signs of vitamin E deficiency. Pharmacol. Rev. 9:1.

Dam, H., and J. Glavind. 1939. Alimentary exudative diathesis, a consequence of vitamin E-avitaminosis. Nature (London) 143:819.

Dam, H., I. Prange, and E. Søndergaard. 1952. Muscular degeneration (white striations of muscles) in chicks reared on vitamin E deficient, low fat diets. Acta Pathol. Microbiol. Scand. 31:172.

Davies, T., J. Kelleher, C. L. Smith, and M. S. Losowsky. 1971. The effect of orotic acid on the absorption, transfer and tissue distribution of α tocopherol in the rat. Int. J. Vit. Nutr. Res. 41:360.

Desai, I. D. 1980. Assay methods. *In* Vitamin E (L. J. Machlin, ed.). Marcel Dekker, N.Y.

Desai, I. D., C. K. Parekh, and M. L. Scott. 1965. Absorption of D- and L-α-tocopheryl acetate in normal and dystrophic chicks. Biochim. Biophys. Acta 100:280.

Desai, I. D., and M. L. Scott. 1965. Mode of action of selenium in relation to biological activity of tocopherols. Arch. Biochem. Biophys. 110:307.

Dickson, J., D. L. Hopkins, and G. H. Doncon. 1986. Muscle damage associated with injections of vitamin E in sheep. Aust. Vet. J. 63:231.

Dinning, J. S. 1962. Nucleic acid metabolism in vitamin E deficiency. Vitam. Horm. 20:511.

Diplock, A. T., E. E. Edwin, J. Green, J. Bunyan, and S. Marcinkiewicz. 1960. Ubiquinones and ubichromenols in the rat. Nature (London) 186:554.

Djaczenko, W., M. N. Urbanowicz, and J. Grabska. 1969. Ultrastructural changes in the Golgi apparatus in the liver of rats with tocopherol deficiency. Acta Med. Pol. 10:415.

Dju, M. Y., K. E. Mason, and L. J. Filer. 1958. Vitamin E (tocopherol) in human tissues from birth to old age. Am. J. Clin. Nutr. 6:50.

Dju, M. Y., M. L. Quaife, and P. L. Harris. 1950. Utilization of pure alpha-, gamma- and delta-tocopherols by laying hens. Am. J. Physiol. 160:259.

Dobbins, W. O. 1975. Human intestinal epithelium as a biological membrane. *In* Pathology of Cell Membranes (B. F. Trump and A. V. Arstila, eds.) pp. 429-453. Academic Press, New York.

Draper, H. H., A. S. Csallany, and M. Chiou. 1967. Isolation of a trimer of alpha-tocopherol from mammalian liver. Lipids 2:4.

Dunkley, W. L., M. Ronning, A. A. Franke, and J. Robb. 1967. Supplementing rations with tocopherol and ethoxyquin to increase oxidative stability of milk. J. Dairy. Sci. 50:492.

Evans, H. M., and K. S. Bishop. 1922. On the existence of a hitherto unrecognized dietary factor essential for reproduction. Science 56:650.

Evans, H. M., G. O. Burr, and T. L. Althausen. 1927. Antisterility vitamin, fat-soluble E. Mem. Univ. Calif. 8:1.

Evans, H. M., O. H. Emerson, and G. A. Emerson. 1936. The isolation from wheat germ oil of an alcohol, alpha-tocopherol, having the properties of vitamin E. Science 64:350.

Fernholz, E. 1938. On the constitution of alpha-tocopherol. J. Amer. Chem. Soc. 59:1054.

Fieser, L. F., M. Tishler, and N. L. Wendler. 1940. The method used for vitamin K synthesis also applicable for the synthesis of a-tocopherol. J. Am. Chem. Soc. 62:996.

Fitch, C. D., and J. F. Diehl. 1965. Metabolism of l-alpha tocopherol by the vitamin E deficient rabbit. Soc. Exp. Biol. Med. Proc. 119:553.

Fridovich, I. 1976. Oxygen radicals, hydrogen peroxide, and oxygen toxicity. In Free Radicals in Biology (W. A. Pryor, ed.), pp 239-277. Academic Press, New York.

Friedman, L., W. Weiss, F. Wherry, and O. L. Kline. 1958. Bioassay of vitamin E by the dialuric acid hemolysis method. J. Nutr. 65:143.

Fromming, K. H. 1984. Moglichkeiten der synthese, probleme der standardiesung und die galenische verarbeitung von vitamin E. Pharm. Ztg. 129:1558.

Gallo-Torres, H. E. 1970. Obligatory role for bile for the intestinal absorption of vitamine E. Lipids 5:379.

Gallo-Torres, H. E. 1982. Transport and metabolism. In: L. J. Machlin (Ed.) Vitamin E. pp 193-267. M. Dekker, New York.

Gallo-Torres, H. E., and O. N. Miller. 1971. Tissue uptake and metabolism of d,l-3-$^3H_2$-α-tocopheryl nicotinate and d,l-α-tocopheryl-1', 2'-$^3H_2$-acetate following intravenous administration. Int. J. Vit. Nutr. Res. 41:339.

Gallo-Torres, H. E., F. Weber, and O. Wiss. 1971. The effect of different dietary lipids on the lymphatic appearance of vitamin E. Int. J. Vit. Nutr. Res. 41:504.

Gibaldi, M., and D. Perrier. 1975. Pharmacokinetics. 1st ed. Marcel Dekker Inc., New York, NY.

Gibaldi, M., and D. Perrier. 1982. Pharmacokinetics. 2nd ed. Marcel Dekker Inc., New York, NY.

Gray, D. E. 1959. Metabolic effects of α-tocopherol acetate. II Influence of α-tocopherol acetate on cholesterol and phospholipid synthesis in rat liver homogenates. Vitam. Horm. 5:19.

Green, J., A. J. Diplock, I. Bunyan, E. E. Edwin, and D. McHale. 1967. Ubiquinone and the function of vitamin E. Nature 19:318.

Griffiths, T. W. 1960. Studies in the requirements of the young chick for vitamin E. The effects of different sources and levels of dietary starch on gain in weight and body vitamin E storage. Brit. J. Nutr. 14:269.

Griffiths, W. T., D. R. Threlfall, and T. W. Goodwin. 1968. Observations on the nature and biosynthesis of terpenoid quinones and related compounds in tobacco shoots. Biochem. J. 5:124.

Guarnieri, C., F. Flamigni, and C. M. Caldarera. 1980. A possible role of rabbit heart cytosol tocopherol binding in the transfer of tocopherol into nuclei. Biochem. J. 190:469.

Gullickson, T. W. 1949. The relation of vitamin E to reproduction in dairy cattle. Ann. N. Y. Acad. Sci. 52:256.

Gyorgy, P., G. Cogan, and C. S. Rose. 1952. Availability of vitamin E in the newborn infant. Proc. Soc. Exptl. Biol. Med. 81:536.

Hakkarainen, J., B. Pehrson, and J. Tyopponen. 1987. Blood vitamin E, selenium and glutathione peroxidase concentrations in heifers fed either on grass or on winter feed. J. Vet. Med. 34:508.

Harris, P. L., and M. L. Ludwig. 1949. Vitamin E potency of alpha tocopherol and alpha tocopherol esters. J. Biol. Chem. 180:611.

Hashim, S. A., and G. R. Schuttringer. 1966. Rapid determinations of tocopherol in macro and microquantities of plasma. Am. J. Nutr. 18:137.

Heaney, D. P., L. Ainsworth, T. R. Batra, P. S. Fiser, A. J. Hackett, G. A. Langford, and A. J. Lee. 1980. A. R. I. Tech. Bull. No. 2, Agric. Can. Anim. Res. Centre, Ottawa, Ontario.

Hidiroglou, M and K. J. Jenkins 1974 Le sort du radiotocopherol administre dans l appareil gastrique ou dans le duodenum du mouton. Ann Biol Anim Bioch. Biophys. 14:667.

Jenkins, K. J., Hidiroglou, M., and A. H Corner. 1972 Control of nutritional muscular dystrophy in lambs by vitamin E implantations. Can J. Anim. Sci. 52:511.

Jenkins, K. J., Hidiroglou, M., J. R. Lessard, Jr., and R. B. Carson. 1970. Metabolism of vitamin E in sheep. Br. J. Nutr. 24:917.

Hidiroglou, M., and K. Karpinski. 1987. Vitamin E kinetics in sheep. Br. J. Nutr. 58:113

Hidiroglou, N., and L. R. McDowell. 1987. Plasma and tissue levels of vitamin E in sheep following intramuscular administration in an oil carrier. Int. J. Vit. Nutr. Res. 57:261.

Hollander, D., E. Rim, and K. S. Muralidhara. 1975. Mechanism and site of small intestinal absorption of a-tocopherol in the rat. Gastroent. 68:1492

Horwitt, M., W. H. Elliot, P Kanjananggulpan, and C. D. Fitch. 1984. Serum concentration of alpha-tocopherol after ingestion of various vitamin E preparations. Am. J. Clin. Nutr. 40:240

Hove, E. L., and P. L. Harris 1947. Relative activity of the tocopherols in curing muscular dystrophy in rabbits. J. Nutr. 33:95.

Hurter, L. R. 1987. A preliminary investigation into the efficacy of intraperitoneal vaccination of sheep and goats kids against heartwater. Onderstepoort J. Vet. Res. 54:507.

Ingold, K. U., G. W. Burton, D. O. Foster, L. D. Hughes, A. Lindsay, and A. Webb 1987. Biokinetics of and discrimination between dietary RRR- and SRR-α-tocopherols in the male rat. Lipids 3:163

Jansson, L., L. Holmberg, and I. Jacobsson. 1981. The effect of dietary gamma tocopheral on serum tocopherals in formula-fed infants. Acta Paed. Scand. 70:297.

Karrer, P., H. Fritzsche, B. H. Ringier, and H. Salomon. 1938. Synthesis of alpha-tocopherol (vitamin E). Nature (London) 141:1057.

Karrer, P., H. Fritzsche, B. H. Ringier, and H. Salomon. 1938. Synthese der α-tocopherols. Helv. Chim. Acta 21:820.

Karrer, P., and O. Isler. 1938 32. U. S. Patent 2, 411, 967.

Farrer, K., and O. Isler. 1941-35. U.S. Patent 2,411,969.

Kasparek, S. 1980. Chemistry of tocopherols and tocotrienols. In Vitamin E: A Comprehensive Treatise (L. J. Machlin, ed.). pp. 7-65. Marcel Dekker, New York and Basel.

Kayden, H. J., and M. G. Traber. 1987. Vitamin E absorption, lipoprotein incorporation and transfer from lipoproteins to tissues. In O. Hayaishi and M. Mino (Eds.) Clinical and Nutritional Aspects of Vitamin E. pp. 129-138. Elsevier Science Publications, New York.

King, J. M., and D. C. Maplesden. 1960. Nutritional muscular dystrophy in calves. Can. Vet. J. 1:421.

Kitabchi, A. E., and J. Wimalasena. 1982. Specific binding sites for D-α-tocopherol on human erythrocytes. Biochim. Biophys. Acta 684:200.

Klatskin, G., and D. O. Molander. 1952. The absorption and excretion of tocopherol in Laennec's cirrhosis. Clin. Invest. 31:159.

Koch-Weser, J. 1974. Bioavailability of drugs. New Engl. J. Med. 291:233.

Krishnamurthy, S., and J. G. Bieri. 1963. The absorption, storage and metabolism of alpha-tocopherol-^{14}C in the rat and chicken. J. Lipid Res. 4:330.

Lannek, N. 1962. The ethiology of nutritional muscular dystrophy in swine. Nord. Veterinaer Med. 14 (Suppl.):2, 19.

Lehninger, A. L. 1975. Biochemistry. The molecular basis of cell structure and function. pp. 302-306. Worth Publishers, Inc., New York, NY.

Leibovitz, B. E., and B. V. Siegel. 1980. Aspects of free radical reactions in biological systems. Aging J. Gerontol. 35:45.

Lundberg, W. O. 1962. Mechanisms. In Symposium on Foods. Lipids and their Oxidation. The Second in a Series of Symposia on Foods held at Oregon State University (H. W. Schultz, E. A. Day, and R. O. Sinnhuber eds.) 2:31-50. Avi Publishing Company, Westport, CT

Machlin, L. J. 1984. Vitamin E. In Handbook of Vitamins: Nutritional, Biochemical and Clinical Aspects. pp. 99-145. Marcel Dekker, New York.

Machlin, L. J., E. Gabriel, and M. Brin. 1982. Biopotency of α-tocopherols as determined by curative myopathy bioassay in the rat. J. Nutr. 112:1437.

Machlin, L. J., J. Keating, J. Nelson, M. Brin, R. Filipski, and O. N. Miller. 1979. Availability of adipose tissue tocopherol in the guinea pig. J. Nutr. 109:105.

MacMahon, M.T., G. Neale, and G. R. Thompson. 1971. Lymphatic and portal venous transport of alpha tocopherol and cholesterol. Europ. J. Clin. Invest. 1:288.

Martin, A. J. P., and T. Moore. 1939. Effects of prolonged vitamin E deficiency in the rat. J. Hyg. 39:643.

Martius, C., and C. Furer. 1963. Ueber die umwandlung des tocopherols im tierkoerper. Biochem. J. 336:474.

Marusich, W. L., G. Ackerman, and J. C. Bauernfeind. 1967. Biological efficiency of d- or dl-α-tocopherol acetate in chickens. Poult. Sci. 46:541.

Marusich, W. L., G. Ackerman, W. C. Reese, and J. C. Bauernfeind. 1968. Relative activity of D and DL-α-tocopheryl acetate based on plasma levels. J. Anim. Sci. 27:58.

Mason, K. E. 1954. The tocopherols. Effects of deficiency. A. In animals. In The Vitamins (W. H. Sebrell Jr. and R. S. Harris, eds.) Vol. III, pp. 514, 541. Academic Press, New York.

Mason, K. E., and P. L. Harris. 1947. Bioassay of vitamin E. Biol. Symp. (Jacques Cattell, ed.). Vol. XII, p. 459.

Mattill, H. A., and R. E. Conklin. 1920. The nutritive properties of milk, with special reference to reproduction in the albino rat. J. Biol. Chem. 44:137.

McMurray, C.H., and W. G. Blanchflower. 1979a. Application of a high-performance liquid chromatographic fluorescence method for the rapid determination of α-tocopherol in the plasma of cattle and pigs and its comparison with direct fluorescence and high-performance liquid chromatography-ultraviolet detection methods. J. Chromat. 178:525.

McMurray, C.H., and W. G. Blanchflower. 1979b. Determination of α-tocopherol in animal feedstuffs using high-performance liquid chromatography with spectrofluorescence detection. J. Chromat. 176:488.

Meadows, J., G. L. Nickell, J. Baxter, and S. Kepple. 1982. Trace substances in environmental health: Proceedings of Univ. of Missouri Annual Conference. Environ. Health 16:268.

McArthur, C. S., and E. M. Watson. 1939. Isolation of alpha- and beta tocopherols and their derivatives. Canad. Chem. 23:350.

McCay, B., J. L. Poyer, M. Pfeifer, E. May, and M. Gilliam. 1971. A function for alpha-tocopherol: Stabilization of the microsomal membrane free radical attack during TPNH-dependent oxidations. Lipids 6:297.

Mellors, A., and M. C. McMarnes. 1966. The distribution and metabolism of alpha-tocopherol in the rat. Brit. J. Nutr. 20:69.

Mervyn, L., and R. A. Morton. 1959. Unsaponifiable fractions of lipid from normal and diseased human kidney. Biochem. J. 72:106.

Muth, O. H., J. R. Schubert, and J. E. Oldfield. 1961. White muscle disease myopathy in lambs and calves. VII. Etiology and prophylaxes. Am. J. Vet. Res. 22:460.

Nair, P. 1972. Vitamin E and metabolic regulation. In Vitamin E and its Role in Cellular Metabolism (P. Nair and H. J. Kayden, eds.). Ann. NY Acad. Sci. 203:53-61.

Nair, P. P., and Z. Lona. 1968. Identification of α-tocopherol from tissues by combined Gas-Liquid-Chromatography, Mass Spectrometry and Infrared Spectroscopy. Arch. Biochim. Biophys. Acta. 127:413.

Nair, P. P., H. S. Murty, and N. R. Grossman. 1970. The in vitro effect of vitamin E in experimental Porphyria. Bioch. et Bioph. Acta 215 21-112.

Newmark, H., W. Pool, J. G. Bauernfeird, and E. Ritter. 1975. Biopharmaceutic factors in parenteral administration of vitamin E. J. Pharm. Sci. 64:655.

Ogihara, T., Y. Nishida, M. Miks, and M. Mino. 1985. Comparative changes in plasma and RBC α-tocopherol after administrations of dl-α-tocopherol acetate and dl-α-tocopherol. J. Nutr. Sci. Vit. 31:164

Oldfield, J. E., J. R. Schubert, and O. H. Muth. 1963. Implications of selenium in large animal nutrition. J. Agr Food Chem. 11:388.

Overman, R.S., J. M. McNeely, M. E. Todd, and I. Wright. 1954. Effects of vitamin E preparations on plasma tocopherol levels. J. Clin. Nutr. 2:168.

Pappenheimer, A. M., and M. Goettsch. 1931. A cerebellar disorder in chicks apparently of nutritional origin. J. Exp. Med. 53:11

Peak, I. R., and J. G. Bieri 1971 Alpha and gamma-tocopherol in the rat: in vitro and in vivo tissue uptake and metabolism. J. Nutr 101:1615.

Pehrson, B., and J. Hakkarainen 1986 Vitamin E status of healthy Swedish cattle. Acta Vet Scand 27:351

Plack, P. A., and J. G. Bieri 1964 Metabolic products of alpha tocopherol in the livers of rats given intraperitoneal injections of ^{14}C alpha-tocopherol. Biochim Biophys Acta 84:229.

Porta, E. A., and W. S Hartroft 1969 Lipid pigments in relation to aging and dietary factors (lipofuscins) In Pigments in Pathology (M Wolman, ed), pp. 191-235. Academic Press, New York

Prasad K. N., D Gaudreau, and J. Brown. 1981. Binding of vitamin E in mammalian tumor cells in culture. Proc Soc Exp. Biol. Med. 166:167.

Pryor, W A. 1970. Free radicals in biological systems Scientific American 223:70

Pryor, W. A 1971. Free radical pathology Chem. Engin. News June 7:34, 41

Pudelkiewick, W. J., and L. D Matterson. 1960. A fat-soluble material in alfalfa that reduces the biological availability of tocopherol 5 methyl-^{14}C-succinate. J Biol Chem. 221:797

Pudelkiewicz, W. J., L D Matterson, L. M. Potter, L. Webester, and E. P. Singsen. 1960. Chick tissue storage bioassay of alpha-tocopherol Chemical analytical techniques and relative biopotencies of natural and synthetic alpha-tocopherol J. Nutr. 71:115

Rammell, C G , and B. Cunliffe. 1983. Vitamin E status of cattle and sheep 2 Survey of liver from clinically normal cattle and sheep for α-tocopherol. N Z Vet J. 31:203

Raychaudhur, C., and I. D Desai 1971 Ceroid pigment formation and irreversible sterility in vitamin E deficiency Science 173:1028

Rigdon, R H., T M. Ferguson, and J Couch 1962 Muscular dystrophy in the chicken A pathology study. Texas Rpts. Biol Med. 20,446.

Roboz, J. 1968. Introduction to Mass Spectrometry, Instrumentation and Techniques pp 1-20 Interscience Publishers, John Wiley and Sons, Inc., New York, NY.

Roles [illegible] Nutr. Rev. 25(4).

Rose, C. S., and P. Gyorgy. 1952. Specificity of hemolytic reaction in vitamin E deficient erythrocytes. Am. J. Physiol. 168:414

Rosenkrantz, H., A. T. Milhorat, and M. Farber. 1951. Counter-current distribution in identification of the tocopheral compounds in feces. J. Biol. Chem. 112:9.

SAS. 1980. A user's guide to SAS. SAS Institute Inc., Raleigh, NC.

SAS. 1985. SAS User's Guide: Statistics. SAS Inst., Inc., Cary, NC

Schwarz, K. 1958. Liver Function. In Symposium on Approaches to the quantitative Description of Liver Function (R. W. Brauer, ed.), pp. 13-24. American Institute of Biological Sciences Pub. No. 4.

Schwarz, K. 1961a. A possible site of action for vitamin E in intermediary metabolism. Amer. J. Clin. Nutr. 9:71

Schwarz, K. 1961b. Discussion article. In The Nature of the Action of Selenium in Replacing Vitamin E. Symposium on the Metabolism and Function of the Fat-Soluble Vitamins A, E, and K (J. G. Bieri, ed.). Amer. J. Clin. Nutr. 9:94.

Schwarz, K. 1962. Vitamin E, trace elements and sulfhydryl groups in respiratory decline: An approach to the mode of action of tocopherol and related compounds. Vitam. Horm. 20:463

Schwarz, K. 1972. In Vitamin E and its Role in Cellular Metabolism (P. Nair and H. J. Kayden, eds.). Ann. NY Acad. Sci. 203:45-52.

Schwarz, K., S. S. Chernick, J. G. Moe, and G. P. Radnan. 1955. A metabolic lesion in dietary necrotic liver degeneration. J. Biol. Chem. 217:829.

Schwarz, K., and C. M. Folz. 1957. Selenium as an integral part of factor 3 against dietary necrotic liver degeneration. J. Am. Chem. Soc. 79:3292.

Scott, M. L. 1962. Vitamin E in health and disease of poultry. Vitam. Horm. 20:621.

Scott, M. L. 1965. Comparative biological effectiveness of d-, dl- and l-forms of α-tocopherol for prevention of muscular dystrophy in chicks. Fed. Proc. 24:901

Scott, M. L., and I. D. Desai. 1964. The relative anti-muscular dystrophy activity of the d- and l-epimers of alpha-tocopherol and of other tocopherals in the chick. J. Nutr. 83:39.

Shiratori, T. 1974. Uptake, storage, and excretion of chylomicra-bound ^{3}H-a-tocopherol by the skin of the rat. Life Sci. 14:929.

Simon, E. J., A. Eisengart, L. Sundheim, and T. Milhorat. 1956a. The metabolism of vitamin E. II. Purification and characterization of urinary metabolites of alpha-tocopherol. J. Biol. Chem. 221:807.

Simon, E. J., E. S. Gross, and A. T. Milhorat. 1956b. The metabolism of vitamin E. I. The absorption and excretion of d-a-tocopheryl 5 methyl C^{14} succinate. J. Biol. Chem. 221:797.

Sieon-Schnoss, R. S., I. A. Reimann, and V. Boehlan. 1964. Vitamin E-therapie: Zur frage der resorption van vitamin E. Notabene Midici 14:793.

Slater, E. C. 1958. The possible role of vitamin E in respiratory-enzyme systems. Proc. of the Fourth Int. Congr. of Biochemistry. Vienna. Vol. XI: Symposium XI. 1 U.B. Symp. Series 13:316.

Sternberg, I., and E. Pascoe-Dawson. 1959. Metabolic studies in arteriosclerosis: I. Metabolic pathway of C^{14} labelled alpha-tocopherol. Can. Med. Assoc. J. 80:266.

Storer, G. B. 1974. Fluorometric determination of tocopherol in sheep plasma. Bioch. Med. 11:71.

Sure, B. 1924. Dietary requirements for reproduction. II. The existence of a specific vitamin for reproduction. J. Biol. Chem. 58:693.

Tappel, A. L. 1962. Vitamin E as the biological lipid antioxidant. Vit. Horm. 20:493.

Tappel, A. L. 1972. Vitamin E and free radical peroxidation of lipids. In Vitamin E and its Role in Cellular Metabolism (P. Nair and H. J. Kayden, eds.). Ann. NY Acad. Sci. 203:12-28.

Thompson, N.J., and M. Hidiroglou. 1983. Effect of large oral and intravenous doses of vitamins D_2 and D_3 on vitamin D in milk. J. Dairy Sci. 66:1638.

Tietjen, G. L., and R. H. Moore. 1972. Some Grubbs-type statistics for the detection of several outliers. Technometrics 14(3):583.

Tikriti, H. H. 1969. The metabolism of vitamin E in the lactating dairy cow in relation to oxidized flavor in milk. Ph.D. dissertation. University of Maryland, College Park, MD.

[illegible], P. H., F. A. Burrows, Jr., A. Weisshaar, and R. L. King. 1968. Utilization of tocopherol-acetate by the lactating dairy cow. J. Dairy Sci. 51:979

Traber, M. G., and H. J. Kayden. 1984. Vitamin E is delivered to cells via the high affinity receptor for low density lipoprotein. Am. J. Clin. Nutr. 40:747.

Trinder, N., C. D. Woodhouse, and C. P. Renton. 1969. The effect of vitamin E and selenium on the incidence of retained placentae in dairy cows. Vet. Rec. 85:550.

United States Pharmacopeia. Twentieth Revision (U.SP XX). 1980. United States Pharmacopeial Convention, Inc. Rockville, MD.

United States Pharmacopeia. 1985. National Formulary. USP XXI. pp 1118-1120.

Vasington, F. D., S. M. Reichard, and A. Nason. 1960. Biochemistry of vitamin E. Vitam. Horm. 18:43.

Weber, F. 1983. Digestion and absorption of nutrients. Int. J. Vit. Nutr. Res. Supp. No. 25:55.

Weber, F., U. Gloor, and O. Wiss. 1962. Fett seife. Anstrichmittel 64:1149.

Wenk, E.F., F. J. Savigne, and E. M. Ellis. 1952. The relative utilization of alpha-tocopherol and alpha-tocopheryl acetate by humans. J. Nutr. 46:353.

Weiser, H., and M. Vecchi. 1982. Stereoisomers of alpha-tocopheryl acetate. II. Biopotencies of all eight stereoisomers, individually or in mixtures as determined by rat resorption-gestation tests. Int. J. Vit. Nutr. Res. 52:351

Whistance, G. R., and D. R. Threlfall. 1967. Biosynthesis of phytoquinones. An outline of the biosynthetic sequences involved in terpenoid quinone chromanol formation by higher plants. Biochem. Biophys. Res. Commun. 28:295

Whistance, G. R., and D. R. Threlfall. 1968. Biosynthesis of phytoquinones. Biosynthetic origins of the nuclei and satellite methyl groups of plastoquinone. Tocopherols and tocopherolquinones in maize shoots, bean shoots and ivy leaves. Biochem. J. 109:577.

Wimalasena, J., M. Davis, and A. E. Kitabchi. 1982. Characterization and solubilization of the specific binding sites for D-α-tocopherol from human erythrocyte membranes. Biochem. Pharmacol. 31:3455.

Wiss, O., R. H. Bunnell, and U. Gloor. 1962. Absorption and distribution of vitamin E in the tissue. Vitam. Horm. 20:441.

World Health Organization (WHO). 1983. Expert Committee on Biological Standardization. 33rd Report. World Health Organization Technical Report Series 687. Geneva.

Yoffey, J. M., and F. C. Courtice. 1956. Lymphatics, lymph, and lymphoid tissue. 2nd Ed, Harvard University Press, Cambridge, MA.

Zalkin, H., and A. L. Tappel. 1960. Studies of the metabolism of vitamin E action. Arch. Biochem. Biophys. 88:113

BIOGRAPHICAL SKETCH

Nicholas Hidiroglou was born in December 1961 in Kapuskasing, Ontario. He attended local schools and graduated from Confederation High School in 1980. He entered Macdonald College of McGill University in September 1980 and was awarded the degree of Bachelor of Science in Agriculture in 1983. In January 1984, the author entered graduate school at the University of Florida and completed a Master of Science degree in animal science in May 1986. He enrolled in a Ph.D. program in 1986 and is currently a candidate for the degree of Doctor of Philosophy in animal science. The author has accepted a Research Associate position at East State Tennessee University in Johnson City.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

L R McDowell

L.R. McDowell, Chairman
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Joseph H Conrad

Joseph H. Conrad
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Douglas B Bates

Douglas Bates
Assistant Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Kermit Bachman

Kermit Bachman
Associate Professor of Dairy Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Rachel Shireman
Rachel Shireman
Associate Professor of Food
Science and Human Nutrition

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August 1989

Jack L. Fry
Dean, College of Agriculture

Dean, Graduate School